543
P91Qf

QUANTITATIVE ORGANIC MICROANALYSIS

F. PREGL

TRANSLATED BY

ERNEST FYLEMAN

THE UNIVERSITY
OF ILLINOIS
LIBRARY

545
P91qEf

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.

To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

JUL 15 1980

L161—O-1096

QUANTITATIVE ORGANIC MICROANALYSIS

THE PLANT ALKALOIDS.

By T. A. HENRY, D.Sc. Second Edition. **28s.**

WAESER'S AIR NITROGEN INDUSTRY.

Translated by E. FYLEMAN, B.Sc., Ph.D., F.I.C.

FOURNEAU'S PREPARATION OF ORGANIC MEDICAMENTS.

Translated by W. A. SILVESTER, M.Sc.

THE BOOK OF RECEIPTS.

By E. W. LUCAS, C.B.E., F.I.C., and H. B. STEVENS, O.B.E., F.I.C. Twelfth Edition. **10s. 6d.**

THE THEORY OF EMULSIONS AND EMULSIFICATION.

By W. CLAYTON. **9s. 6d.**

CLOUDS AND SMOKES:
The Properties of Disperse Systems in Gases.

By W. E. GIBBS, D.Sc. **10s. 6d.**

J. & A. CHURCHILL

QUANTITATIVE ORGANIC MICROANALYSIS

BY

FRITZ PREGL

D.Sc., PH.D.

Professor of Medicinal Chemistry; Principal of the Medico-Chemical Institute of the University of Graz; Corresponding Member of the Academy of Sciences, Vienna

TRANSLATED FROM THE SECOND REVISED AND ENLARGED GERMAN EDITION

BY

ERNEST FYLEMAN

B.Sc., PH.D., F.I.C.

Chief Chemist, Messrs. J. F. Crowley & Partners, Consulting Engineers, London

WITH 42 ILLUSTRATIONS

LONDON

J. & A. CHURCHILL

7 GREAT MARLBOROUGH STREET

1924

Printed in Great Britain

545
P91pEf

UNIVERSITY OF ILLINOIS
LIBRARY-CHEMISTRY

Sept. '25 K.P.

PREFACE TO FIRST EDITION

For many years I have preferred personal instruction in the laboratory to those anxious to acquire knowledge of the subject. Finally, the time appeared to have arrived to publish a book on the whole field of organic microanalysis. For a long time I resisted the desire of many colleagues and friends to communicate my work in print, in addition to my lectures, as of the two methods of publication open to me the one which appeared to me most useful, and most satisfactory to me personally, was by a series of individual publications corresponding to the position attained from time to time. As the better is always the enemy of the good, and always will remain so, it appeared to me more advantageous not to publish my communications in the form of a monograph until the methods which had been studied had not only been tested and revised with regard to all the fundamental conditions, but had also proved themselves in practice during many years.

In writing this book I could not avoid a certain broad attention to detail which might repel some readers, as I wished to include the trains of thought which had led to the development of the methods; it may, however, be mentioned, in order to reassure such readers, that the apparent difficulties are so slight that organic microanalysis may be acquired by anybody.

In the production of this book I have received help from many sources. Dr. Lieb assisted me particularly in reading the proofs. Mr. Hermann Seidl produced most of the illustrations, and the publisher assisted me to the utmost in every way. I here express my thanks to all these gentlemen.

FRITZ PREGL.

Chemistry Research 18 Je 25 Bucket Ed. 2.

580174

PREFACE TO SECOND EDITION

WITHIN barely five years the whole of the first edition of this book has been disposed of in spite of the Great War and its consequences. The economies achievable by microanalytical methods, both in the required quantities of material and gas, and also in time and space, and the readiness with which analyses can be duplicated, have long been recognised. The altered conditions since the war emphasise these economic considerations and thus favour the spread of microanalysis. I have therefore much pleasure in accepting the invitation of the publisher to issue a second edition in which a number of innovations, which I believe to be very valuable, have been incorporated.

I have once more pleasure in acknowledging the assistance which has been rendered to me by the publisher. I have been greatly helped in reading the proofs by Professor Hermann Krauss, to whom my best thanks are here due.

FRITZ PREGL.

TRANSLATOR'S PREFACE

A CHARACTERISTIC feature of modern research in organic chemistry, whether of an industrial or purely scientific nature, is the increasing attention which is given to compounds present in relatively minute amount, and a recognition of the important part which they may play. The importance of compounds present in such small quantities is particularly obvious in connection with the study of bio-chemical phenomena ; under the name of enzymes, vitamines, hormones and so forth, they influence chemical and vital processes to an extent out of all proportion to the amounts in which they are present, and this is equally true in other fields. Many a works difficulty or erratic occurrence during large scale industrial operations is ultimately found to be due to the unexpected presence of some obscure compound in very small quantity. The isolation of such compounds is a tedious and costly operation, and methods of analysis of such materials, which can be practised on minute amounts, fulfil a very serious requirement. In times gone by, many workers have had the experience of successfully isolating perhaps one or two decigrammes of material or even less, by the exercise of almost unlimited patience and a very large amount of time, and have been confronted with the impossibility, as it then seemed, of establishing its composition.

By the classical methods of elementary organic analysis, with which the names of Liebig, Dumas, and Carius will always be associated, and which have undergone relatively little change until the turn of the present century, a reliable set of analytical data for a carbon compound containing several elements, was hardly attainable unless 0·5 to 1 gm. were available. By Professor Pregl's methods, 12 to 15 mgm. suffice for this purpose, and, if a few further milligrammes are available, a molecular weight determination may be included. These results, and the simplicity and elegance of the methods by which they have been attained, have well deserved the world-wide recognition which has been accorded to Professor Pregl's work, and no further explanation of

the desirability of the translation of his book into English is really necessary.

The translator has endeavoured to make his translation as literal as possible so far as the difference in the two languages allows. Manipulative and other details are described with a minuteness to which the average English worker is unaccustomed. This feature is preferable in a book of this character to the scant attention to detail which characterises much modern work. It is clear that without extensive devotion to experimental details, work of this delicate character would be impossible.

ERNEST FYLEMAN.

CONTENTS

PAGE

I. INTRODUCTION 1

II. KUHLMANN'S MICROCHEMICAL BALANCE AND THE USE OF THE SAME 7

History. Construction. Capabilities and applicability. Rules for weighing. Erection. Effects of surrounding conditions. Rules for zero point deviations. The weights and other objects which are kept in the balance case. Objects which are not kept in the balance case. "Acclimatisation." Cleaning and adjustment of the balance. Weighing with counterpoises.

III. THE DETERMINATION OF CARBON AND HYDROGEN IN MINUTE AMOUNTS OF ORGANIC MATERIAL 15

Historical Introduction 15

Oxygen, Air and Delivery Tubing for the same . 17

The Pressure Regulator 18

Construction, purpose and manipulation. The U-tube with bubble-counter. Filling, use and calibration of the same.

Materials for Filling the Combustion Tube for Carbon and Hydrogen Determinations . . 23

The tube itself. The silver wool. Asbestos for the Gooch crucible. Lead peroxide and lead peroxide asbestos. Copper oxide and lead chromate.

Filling the Combustion Tube for the Carbon and Hydrogen Determination 25

Detailed explanations of the choice of filling. Trichlor-nitro-benzene as a suitable material for comparing the value of various tube fillings.

The Heating Mortar and the Combustion Stand . 31

Construction, purpose and manipulation of the heating mortar. Erection of the complete apparatus for the carbon and hydrogen determinations.

The Absorption Apparatus 35

Historical development from the earliest form to the present construction of absorption tubes of constant weight with ground joint. Their filling and manipulation. Their properties with regard to constancy of weight.

PAGE

THE MARIOTTE FLASK. 42

Construction.

THE CONNECTING TUBING 43

Its defects and their partial rectification by impregnation.

ON THE PRESSURE AND SPEED RELATIONS WHICH ARE NECESSARY IN CARRYING OUT THE ANALYSIS . . 45

The pressure diagram of the whole system. Rules for use of the Mariotte flask. Combustion without Mariotte flask. Summary on the essential features of the apparatus.

BLANK TESTS 51

The importance of equalisation of the calcium chloride in the absorption tubes.

VARIOUS CONSIDERATIONS ON THE INFLUENCE OF DEFECTIVE RUBBER TUBING CONNECTIONS ON THE ANALYTICAL RESULTS UNDER VARIOUS CONDITIONS, AND ON THE RELATIONS BETWEEN PRESSURE, VELOCITY OF THE GAS CURRENT AND THE CONSTRUCTION OF THE ABSORPTION TUBES 53

The three most important conditions for ensuring accuracy in microanalytical determinations of carbon and hydrogen : (1) Equalisation of the calcium chloride ; (2) Ensuring a time of contact of 3 to 4 minutes for each particle of vapour which passes ; (3) The avoidance of erroneous increases or losses of weight.

THE PREPARATION OF MATERIALS FOR ANALYSIS . . 58

(A) SOLID SUBSTANCES 58

Weighing into the boat. Drying on the drying block at ordinary pressure and in the micro-desiccator at reduced pressure.

(B) LIQUIDS 62

Weighing the liquid in suitable capillaries.

THE COURSE OF THE ANALYSIS 65

Ash free materials. Inorganic residues.

THE COURSE OF THE DETERMINATION OF CARBON AND HYDROGEN WITH THE USE OF A MERCURY GAS-HOLDER 69

The method and its use.

IV. GAS-VOLUMETRIC DETERMINATION OF NITROGEN IN SMALL AMOUNTS OF ORGANIC MATERIAL (MICRO-DUMAS METHOD) 72

Historical and theoretical introduction.

PAGE

The Kipp Generator for Production of Carbon Dioxide 79

The Filling and Preparation of the Combustion Tube for the Gas-Volumetric Determination of Nitrogen 83

The Precision Microazotometer 85

Fifty per Cent. Caustic Potash Solution . . 87

Preparation of the Material for the Determination of Nitrogen as Gas 88

The Course of Analysis of Solid and Liquid Substances 89

V. THE DETERMINATION OF NITROGEN BY KJELDAHL'S METHOD IN SMALL AMOUNTS OF MATERIAL (MICRO-KJELDAHL METHOD) . 94

The apparatus and its manipulation. The N/70 normal solutions and titration with methyl-red. The apparatus. Parnas and Wagner.

VI. THE MICROANALYTICAL DETERMINATION OF THE HALOGENS AND OF SULPHUR . . . 102

History of the evolution of the new process, which depends on the destruction of the organic material in a stream of oxygen.

The Combustion Tube with Bead-filling. . . 104

The Two Platinum Contacts 105

The Filter Tubes 106

Reagents 109

Distilled Water 109

Concentrated Nitric Acid 110

Sodium Carbonate Solution Free from Halogen . 110

Bisulphite Solution Free from Halogen . . 111

Silver Nitrate Solution 112

The Course of the Halogen Determination . . 112

The Course of the Determination of Sulphur . 118

The Determination of Sulphur by Titration in Substances Free from Nitrogen and Halogens . . 124

Some Analytical Results. 124

Halogen determinations. Sulphur determinations.

The Determination of Sulphur and of Halogens in Small Quantities of Material by Carius' Method . 125

PAGE

VII. THE MICROANALYTICAL DETERMINATION OF PHOSPHORUS AND ARSENIC IN ORGANIC SUBSTANCES (DESCRIBED BY DR. HANS LIEB) 127

(A) Phosphorus. 127

The utilisation of the precipitate of ammonium phosphomolybdate and the relation of its weight to the weight of the phosphorus which it contains. Conditions for the production of the precipitate. The combustion of the organic material with sodium carbonate and sodium nitrate in a stream of oxygen. The course of the determination.

(B) The Microanalytical Determination of Arsenic. 133

VIII. THE DETERMINATION OF METALS IN SALTS. . 135

IX. QUANTITATIVE MICRO-ELECTRO-ANALYSIS . . 138

Description and manipulation of the necessary apparatus. The quantitative determination of copper in preserved vegetables and their preliminary treatment for the purpose.

The Determination of Copper in Preserved Vegetables 144

X. THE MICROANALYTICAL DETERMINATION OF CARBOXYL 148

XI. THE MICROANALYTICAL DETERMINATION OF METHOXYL AND ETHOXYL 150

Description and manipulation of the apparatus for the determination of methoxyl.

Preparing the Material for the Methoxyl Determination 154

Red Phosphorus 155

Hydriodic Acid. 156

Alcoholic Silver Nitrate Solution 156

Some Analytical Results of the Micro-Determination of Methoxyl 157

XII. THE MICROANALYTICAL DETERMINATION OF METHYL GROUPS ATTACHED TO NITROGEN 158

Construction and manipulation of the necessary apparatus. Course of the determination both gravimetrically and titrimetically.

Some Analytical Examples of the Microanalytical Determination of the Methylimido-Group . . 162

PAGE

XIII. MOLECULAR WEIGHT DETERMINATIONS IN SMALL QUANTITIES OF ORGANIC SUBSTANCES BY DETERMINATION OF THE RISE OF BOILING POINT 164

The small Beckmann thermometers of Siebert and Kühn. The principle of heating by means of a uniform stream of pre-heated air passed round the boiling vessel. The necessary apparatus and its manipulation. The tablet press and the preparation of tablets. The course of a determination.

Molecular Weight Determinations 171

XIV. NOTE ON THE PURIFICATION OF SMALL AMOUNTS OF MATERIAL 172

Crystallisation with the use of a micro-filter and of a micro-vacuum filter. The distillation of minute amounts of liquid *in vacuo*. The sublimation of minute amounts of material at ordinary pressure and *in vacuo*.

XV. CALCULATION OF THE RESULTS OF MICRO-ANALYSES 176

Conversion tables with factors and the mantissæ of the logarithms. Multiples of the methoxyl, ethoxyl, methyl and carboxyl groups, and the mantissæ of their logarithms.

Calculations of Molecular Weight when Using 1·5 c.c. of Di-Ethyl Ether, Acetone, Chloroform, Ethyl Alcohol, Benzene, Water and Glacial Acetic Acid 178

XVI. LIST OF SOME RESEARCHES CARRIED OUT WITH THE HELP OF THE MICROANALYTICAL METHODS DESCRIBED IN THIS WORK 179

XVII. FINAL REMARKS. PROGRAMME OF STUDIES FOR BEGINNERS. SOURCES OF SUPPLY OF APPARATUS AND CHEMICALS 184

INDEX 187

QUANTITATIVE ORGANIC MICROANALYSIS

I. Introduction

In the course of a lengthy investigation in the summer of the year 1910, I obtained a fission product which was only obtainable at the time in extremely small quantity. Thus a decision had to be arrived at, either to continue the investigation with exceptionally large quantities of the original material or so to modify the quantitative analysis of organic substances that it should be possible to obtain correct analytical figures with quantities of material hitherto unheard of, so that formulæ could be determined with certainty. I had more particularly in view the necessity for working out a quantitative microanalytical method for the determination of carbon and hydrogen and also of nitrogen by measurement of the gas volume.

As no experimental material of this kind existed, it appeared to me all the more interesting to work at this entirely neglected problem. I was encouraged by the fact that Emich had already shown the essential reliability and advantages of working with small amounts of material in various inorganic determinations; this was a factor which enabled me to attack the problem which I had set myself with great confidence, in spite of the difference of the objective.

By the end of 1911 this problem had been essentially solved; the methods elaborated by that date, and described in detail in E. Abderhalden's "Handbuch der biochemischen Arbeitsmethoden," vol. 5, pp. 1307–1356, enabled carbon and hydrogen to be determined in 7 to 13 mgm. of organic material even in the presence of nitrogen and of small amounts of halogen and sulphur; also of nitrogen in 4 to 8 mgm. of material, either by measurement of the gas volume or alkalimetrically, and the determination of sulphur and halogen, also in 4 to 8 mgm. of material by Carius' method. Thus the fundamental applicability of elementary organic microanalysis had been proved for a series of elements.

although various observed effects awaited explanation and required further modification of the methods.

In this direction the years 1912 and 1913 were very fruitful. In the determination of carbon the necessity for passing the gases through the combustion tube twice was rendered unnecessary by increasing the tube filling and replacing the mercury gas holder by a Mariotte flask. The quantity of material required was successfully reduced to 2 mgm. by the discovery of a series of hitherto unnoticed effects, for instance, recognition of the fact that new india-rubber tubing led to serious contamination of the gases passing through it ; and by the determination of the necessary conditions for the complete decomposition of the organic material, and their quantitative expression with regard to the velocity of the gases and time of contact with the heated filling of the combustion tube, instead of the ambiguous terms " rapid " and " slow." The influence and efficiency of various fillings for the combustion tube were also investigated, more particularly in the case of substances containing much halogen and sulphur.

The origin of the admixture of other gases in the determination of nitrogen by the gas volume, which had hitherto necessitated a deduction of 10 per cent. of the actual reading, was discovered, and methods of preventing their formation were found. An essentially new method of determining halogen and sulphur was also developed, based on the complete destruction of the organic material by combustion in a stream of oxygen. Practical requirements led to the discovery of a microanalytical method of determining copper electrolytically and also of determining molecular weight by rise of boiling point. A method of microanalytical determination of methoxyl was also elaborated during this period, together with initial attempts at the determination of methyl attached to nitrogen.

During these investigations I was very greatly assisted by my co-workers, namely, by my former assistants, Dr. Max de Crinis and Dr. S. Edlbacher, also by Dr. Heinrich Poda, Dr. Emil Schwinger, of Graz, Dr. Johann Dubsky, of Zürich, and more particularly by my present assistant, Dr. Hans Lieb. At a low and superficial estimate the experimental material for working out methods of organic microanalysis necessitated about 10,000 exact weighings.

Already during my Innsbruck activities (1910–13) much interest was shown in microanalysis by various well-known colleagues, more particularly by Professor G. Goldschmiedt, who

gave much friendly assistance until his death, and whose help I remember with gratitude. My methods were introduced by him at his Institute in Vienna in the year 1912, and after that date were studied and used by his assistants, Dr. E. Philippi and Dr. Bregant, and also by himself and his pupils. I have pleasant memories of the years which I spent in joint work with my Innsbruck colleague, Carl Brunner. My thanks are also due to Geh-Rat. Albrecht Kossel, of Heidelberg, and to my colleague, Fritz Strauss, of Strassburg, whom I received as guests at my Innsbruck Institute, together with many others.

At the Naturforscherversammlung in Vienna (September, 1913) I had the opportunity of expounding the main features of organic microanalysis, which were by then established, in an experimental lecture. The consequence was that from that date until the commencement of the Great War a number of colleagues acquired the technique in my Institute. I may mention in this connection the following in chronological order :

Dr. Eckert (Prague).
Dr. F. Hansgirg (Graz).
Dr. Birkenbach (Ludwigshafen).
Mr. Kolisko (Vienna).
Dr. Schranz (Leverkusen).
Dr. Brigl and Dr. Neber (Tübingen).
Dr. Virgin (Upsala).
Professor Franzen (Karlsruhe).
Dr. Schrader (Heidelberg).
Dr. Yllner (Stockholm).
Dr. Sernagiotto (Bologna).
Dr. Cornubert (Sorbonne, Paris).

These circumstances provided me, assisted by Dr. Lieb, with ample opportunity to discover which precautions and manipulations could be generally recommended and which not, and in this way the experimental material was effectively increased. By these means knowledge of organic microanalysis was spread and found adherents without publication by me of the experience which had been gained, as I avoided publicity until full assurance had been obtained that each of the methods recommended was accurate, apart from unknown imperfections which might have been hidden by compensating errors, and that everything which had appeared to require it had found a satisfactory explanation ; for a large series of analytical data in agreement with theory is no proof of the reliability of an analytical method until all the

necessary fundamental conditions have been realised, tested and established. Our experience was considerably increased by the fact that Dr. Hans Lieb willingly undertook to carry out the most various microanalyses for large numbers of colleagues ; this gave me the opportunity, not only to investigate substances of very varied constitution, but also to investigate numerous cases in which all manner of disturbing phenomena were met with and thus to learn how to overcome them.

Thus the microanalytical processes which will be described do not rest merely on theoretical considerations and occasional experiments, but have been elaborated by actual practical experience, and may, therefore, claim to be adapted to practical purposes.

In the nature of the case it is clear that the number of those who utilise organic microanalysis in the course of their work will increase after the Great War, and that the subject will extend its scope beyond purely scientific laboratories, as various methods of technical investigation will be greatly benefited by microanalysis and by the economy in time and material which it offers. The first steps in this direction have already been made. The electrolytic determination of copper in preserved vegetables has been successfully elaborated, and Dr. Ripper, Director of the Agricultural Testing Station in Görz, has already successfully used microanalysis for the investigation of wine ; in this connection he has worked out a microanalytical method for the determination of glycerine, which is simple and accurate, in contrast to our present official method of determining that substance.

It will give me the greatest satisfaction if this new subject of organic microanalysis, after successfully and quickly passing through all its juvenile ailments, should become more and more important in the course of its later life. I shall always be grateful for any serious work which conduces to this end.

Although the Great War and the following years were not favourable to the further development of organic microanalysis, the interest shown by us and by many colleagues has not subsided. This is shown by the fact that during this unfavourable period the first edition of this book has long been out of print, and that in the course of years many more persons have enrolled themselves for instruction in my Institute than it was possible to accommodate, in addition to the increasing requirements of normal teaching routine.

The fact that organic microanalysis has been adopted in the

most various scientific laboratories by the large number of those who were trained by us since the appearance of the first edition of this book shows that interest in it has been maintained. Were I to mention all those who have been instructed in my laboratory, and all the laboratories in which microanalysis is now employed, their enumeration would widely exceed the limitations of this book. Of the latter I will only mention, in chronological order, the following :

Physiological Institute of the University of Heidelberg (Professor A. Kossel) ; Chemical Institute of the University of Upsala (Professor Widmar and Professor Ramberg) ; Chemical State Laboratory in Munich (Professor Willstätter) ; Chemical Institute of the Technical High School in Munich (Professor Wieland) ; Kaiser Wilhelm Institute for Investigations on Coal at Mühlheim-on-Ruhr (Professor Fischer) ; Scientific Laboratory of the firm of Merck at Darmstadt ; Chemical Institute of the University of Freiburg i. B. (Professor Wieland) ; Chemical Institute of the University of Stockholm (Professor von Euler) ; Physiological-Chemical Institute of the University of Würzburg (Professor von Ackermann).

Of the new investigations which have been made by me and my fellow workers at my Institute since the appearance of the first edition, I may mention, as especially valuable, the use of lead peroxide asbestos, the micro-muffle for the simplified determination of ash in a boat, improvements in the production of reagents free from halogen for the halogen determination, the volumetric determination of carboxyl, and also the gravimetric determination of arsenic in organic substances, elaborated by Dr. Lieb and Dr. Zima, the manipulation of low boiling liquids in the determination of carbon and hydrogen, the experiments on the advantages and disadvantages of the acidimetric and iodometric methods of determining ammonia by Kjeldahl's method, and finally, a large number of not unimportant observations which were mainly made in my Institute or collected by me. Finally, I wish to point more particularly to the investigation of the question whether microanalysis, more particularly that of organic substances, involves difficulties in accurate sampling which may restrict its use for investigating solid materials. Dr. Benedetti-Pichler came to the conclusion that a true average sample can be obtained which is accurate within the permissible error of the analytical methods. He found this to be the case by preparing an average sample by the customary methods after first thoroughly

crushing the material in a porcelain mortar and, if necessary, subsequently grinding harder material in an agate mortar for five minutes, using a total quantity of 1 gm. and taking a sample of 3 to 5 mgm. The results were controlled by mathematical considerations, and also by practical analytical determinations in several artificial mixtures, and by determinations of the readily attainable grain size.

It is clear that a new development, such as was the introduction of organic microanalysis at the time of the appearance of the first edition of this book, must stimulate developments and modifications in wide circles, and thus it happens that in the course of the last few years several suggestions of this kind have appeared in the literature. I have discussed such of these as appeared to me to constitute an improvement and which I could especially recommend, in accordance with what I expressed in the first edition, to the effect that I would thankfully avail myself of any contributions to this new subject. I have also thought it necessary to express my objections to those developments which I consider less desirable or even objectionable.

I append a bibliography of the older and newer literature without any attempt at completeness :

Fritz Pilch. Monatshefte f. Chemie (1911), **32,** 26.
Dr. W. Brunner. Inaug.-Diss. (Freiburg, 1914.)
Eckert, in H. Mayer's Analyse u. Konstitutionsbestimmung, 3rd edition, pp. 918–994.
Ivar Bang. Methoden zur Mikrobestimmung einiger Blutbestandteile. (Wiesbaden, 1916.) (I. F. Bergmann.)
Dubsky, in Chem.-Ztg. (1916), **40,** 201 ; Vereinfachte quantitative Mikroelementar-analyse organischer Substanzen (1916–17), published by Veit & Co., Leipzig ; Ber. (1917), **50,** 1711 ; Helv. chim. acta (1919), **2,** 63, 76 ; Die ersten Entwicklungsjahre der organischen Makro- und Mikro-Elementaranalyse, Chem. Weekbl. Deel (1919), **16,** 1482–1493 ; Weyl. Organische Arbeitsmethoden, 2nd edition (1919), p. 149.
E. Abderhalden and A. Fodor. Zeitschr. f. physiol. Chemie (1917), **98,** 190.
S. Edlbacher. Zeitschr. f. physiol. Chemie (1918), **101,** 278–287.
R. Strebinger, Chem.-Ztg., Nos. 8 & 17.
E. Muller and H. Willenberg. Journ. f. prakt. Chemie (1919), **99,** 34–44.
Schoeller. Zeitschr. f. angew. Chemie, **34,** No. 93, pp. 581–583 ; No. 94, pp. 563–587.
F. Wrede. Ber. (1922), **55,** 557–563.
F. Holtz. Ber. (1922), **55,** 1496–1497.
Emich. Handb. d. biol. Arbeitsmethoden (1921), Div. I., Part 3, H. 1.
H. Lieb. Handb. d. biochem. Arbeitsmethoden (1919), Vol. 9 ; Handb. d. biol. Arbeitsmethoden (1921), Div. I., Part 3, H. 2, 3 and 5–16.
Ripper and Wohack. Zeitschr. f. d. landwirtschaftl. Versuchswesen in Osterreich, **19,** 372 ; **20,** 102.
Wohack. Handb. d. biol. Arbeitsmethoden, Div. I., T. 3, H. 3, 7, 25.
Parnas and Wagner. Biochem. Zeitschr.
Benedetti-Pichler. Ztschr. f. analyt. Chemie, **61,** 305–331.

II. Kuhlmann's Microchemical Balance and the use of the Same

The exceptional achievements of W. Kuhlmann in the construction of balances were already well known to me at the time when he managed the works of Paul Bunge, of Hamburg, after the latter had already lost his eyesight, for in the nineties of last century a short-armed balance constructed by him was at my disposal. At a later period I worked for years exclusively with an analytical short-armed balance (4B of his catalogue), and became so well acquainted with the advantages of Kuhlmann's

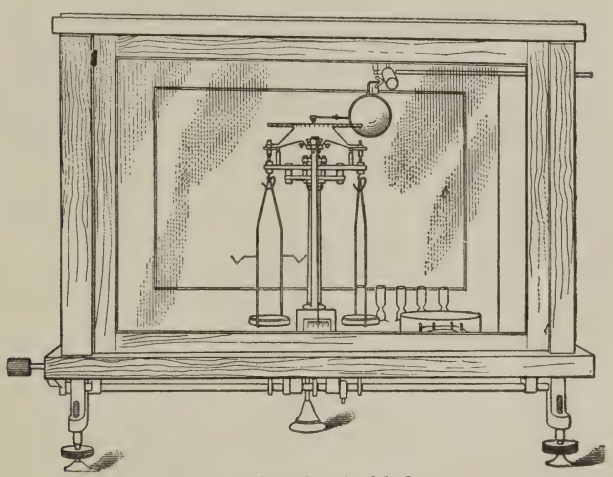

FIG. 1.—Microchemical balance.

construction that I disliked to work with other balances. Already at that time Kuhlmann produced "Assay Balances for Precious Metals" for a maximum load of 20 gm. Emich, at whose laboratory I first saw these, mentions them in his lecture [1] on microchemistry as an example of the fact that there are balances which weigh accurately to within 1 to 2 hundredths of a milligramme, and suggests their use in carrying out the so-called "centigramme method." In further communications by Emich and Donau, this balance is also alluded to as "Kuhlmann's small analytical balance." Its capabilities and manipulation were described by Donau in "Die Arbeitsmethoden der Mikro-chemie," 1913, pp. 42–44.

This assay balance was the predecessor of the modern micro-

[1] *Berichte* (1910), **43**, p. 29. See also *Monatsh. f. Chemie* (1908), vol. 29, p. 1082, Illustration 1, Microchemical balance.

chemical balance which Kuhlmann constructs to my specification for the purposes of quantitative microanalysis.[1]

This microanalytical balance, with a beam only 70 mm. in length and a maximum load of 20 gm., has a constant sensitiveness whether loaded or unloaded. This unusual property is due, on the one hand, to the circumstance that the three-knife blades are perfectly straight, lie in one plane, and are parallel to one another, and on the other hand to the rigid construction of the beam, which shows no detectable distortion even at maximum load. The balance is always provided with a rider adjustment carrying a lens, a counterpoised sliding front, hooks for carrying absorption apparatus on the left balance pan, and also with a vane with small regulating screw for zero adjustment, which is attached independently of the regulation of the sensitiveness. As a consequence of the improvement of methods of grinding the knife edges, due to Kuhlmann, combined with the determination by me of the necessary manipulative precautions and adjustments, it is now readily possible with this instrument to weigh with an accuracy of $\pm$ 0·001 mgm. a maximum load of 20 gm. The sensitiveness therefore amounts to one in ten millions = 10^{-7}. It is, perhaps, not too much to say that this represents the limit of possible achievement in the construction of balances of precision.

It is an extraordinary fact that with careful use this sensitiveness is preserved for years. Only after eight or ten years do symptoms of ageing occur in consequence of hardly appreciable wear of the knife edges, which shows itself by more rapid "fatigue" and also by reduction of the sensitiveness by, at most, one-half. It is obvious that careless handling would lead to these results more quickly.

The microchemical balance has a wider range of use than any other instruments known to-day which are sensitive to differences of weight of 0·001 to 0·002 mgm., for none of the others can carry 20 gm., but at most 5 to 10 gm., and they are, therefore, not as independent as is the microchemical balance of the form and weight of the object to be weighed. With the microchemical balance we can weigh any substance up to a weight of 20 gm.

[1] I wish to add that Mr. Kuhlmann has also solved other problems with similar success. He has produced the best automatic sorting machines for coins and also automatic registering analytical balances for showing loss and increase of weight. See Dr. Anton Munkert, "Münzplatten-Sortiermaschinen," in Dingler's *Polytechnische Journal* (1907), 48th year, vol. 322; also E. Abderhalden in *Skandinavische Archiv. für Physiologie* (1913), vol. 29, pp. 75–83, with 3 plates; and in *Zts. f. Fermentforschung*, (1915), vol. 1, section 2, pp. 153–164; section 3, pp. 229–232.

of any shape so long as it can be stood, laid, or hung on to the balance pan.

It is a special feature of Kuhlmann's balances, both of the microchemical balance and of the larger models, of which 4A and 4B are used in many laboratories as analytical short beam balances, that their sensitiveness is constant at all loads, that is, whether the pans are empty or loaded with the maximum load. A displacement of the rider by one tooth of the rider scale always causes a deflection of ten divisions on the pointer scale of the balance in question. In the microchemical balance the rider scale consists of 100 uniform notches, cut by a dividing engine in such a manner that the rider is forced into the lowest point of the notch, particularly if it is caused to oscillate by a slight push to one side. A 5 mgm. rider is used with the microchemical balance, and as the balance is so arranged that it is in equilibrium when unloaded and when the 5 mgm. rider is in the first notch on the left, over the left pan suspension, a displacement of the rider to the one hundredth notch, which is over the right pan suspension, corresponds to a load of 10 mgm. in the right pan. A rider displacement of ten notches, therefore, corresponds to a change of load of 1 mgm., and thus the numerals stamped into the beam below each tenth notch indicate whole milligrammes. These numerals start from 0 below the first notch and increase continuously to the right ; therefore a displacement of the rider by one notch to the right corresponds to an increase of load of 0·1 mgm. on the right pan of the balance, and this corresponds to an alteration of the equilibrium position of ten divisions on the pointer scale which is magnified by the use of the mirror. It follows, further, that one division of the pointer scale corresponds to 0·01 mgm., and as the individual oscillations of the swinging pointer can be read to one-tenth of a division after very little practice, it follows that if all the necessary conditions are observed one can weigh with an accuracy of $\pm$ 0·001 mgm. Difficulties only occur with observers with abnormal eyesight, particularly with those who are astigmatic or seriously myopic, particularly if these defects are not properly corrected. There is also the further disadvantage that such observers tend to bring their heads too near to the balance and influence the regularity of the oscillations by their breathing.

In carrying out such delicate weighings it is advisable to record deflections by considering the middle point of the scale as zero and each tenth part of a division as unity ; for instance, a deflec-

tion of 2·7 scale divisions to the right is described as " 27 right," and a following deflection of 3·4 scale divisions to the left as " 34 left." The mean deflection from the zero point is in this case " 7 left," that is, one must subtract 0·007 mgm. from the sum of the weights on the right hand pan and the weight corresponding to the rider reading. If in the example which has just been mentioned the observed deflections to the right and to the left were interchanged, the mean deflection would be " 7 right," that is, one would have to add 0·007 mgm. to the sum of the weights present on the right balance pan and that indicated by the rider. It is also advisable to subtract two consecutive readings from one another mentally if they are on opposite sides of the zero point and add them similarly if they are both to the left or to the right of the scale. Beginners will find it necessary to note the individual observations on paper for a few days only. Later one becomes accustomed to carrying out the operation mentally and almost automatically. This is rendered easier by the fact that Kuhlmann's microchemical balance has the advantage, on the one hand, of such minute and regular reductions in the amount of swing (small fatigue) that this enables the necessary calculations to be carried out with ease, and also that the mean swing is so constant in amount that the results of such calculations are almost always identical, and that it is very rarely, perhaps once or twice in six times, that they differ by one unit, that is, by a tenth part of a division, corresponding to 0·001 mgm.

This method of observing the swing enables one to test the accuracy of the weighing at any time, for it is clear from the above that a deflection to the right positive side of the scale must be added to the sum of the weights and of the rider reading, and if to the left must be subtracted from this sum. If we therefore displace the rider, after a positive deflection has been obtained, by one notch to the right, the deflection will become negative and be to the left, and this deflection, when added to the former positive deflection, should result in a value of 100. In this manner one can also check the correct adjustment of the sensitiveness of the balance, both when unloaded and at maximum load, without difficulty.

Suitable support of the balance is particularly important. It has been found best to use a marble bracket, supported on iron bearers which are mounted in the wall. Strips of sheet lead, placed between the marble plate and the iron, are best for absorbing vibration. In choosing a locality for the erection of the

balance, the lighting and heating arrangements of the room are more important than freedom from vibration, for, although the microchemical balance is little effected, if at all, by the passage of a tramcar in the vicinity, it is very sensitive to air currents, and more so to such within the balance case than to any which may be present in the room. Therefore it is inadvisable to mount the balance in front of a wall within which there is a heating unit. It must not be placed near a stove, but may quite well be placed on the opposite wall. It must never be exposed to direct sunshine and one should avoid the presence of a lighting unit in the immediate neighbourhood. All the above-mentioned influences cause deflections of the zero point which, if they are small and tend to a constant value, such as the change from daylight to artificial light, do not affect the accuracy of the weighings. I have recently completely eliminated the effect of the lighting by installing in our balance room a 600 candle-power half-watt lamp, the light of which was reflected from the ceiling. Professor von Euler, of the University of Stockholm, ensured a high degree of insensitiveness to artificial light by placing round the marble bracket and balance an enclosure of glazed walls and doors reaching from the floor to more than the height of the observer, and placing an electric lamp for illuminating the balance immediately above the glass roof of this enclosure.

If one is unable to find a place for the erection of the balance which is sufficiently favourable completely to avoid variations of the zero point, these must be taken into account, more particularly in the case of drying operations, which may last for hours or days. I have found the following practical rules useful in calculating the consequent changes of weight :

1. The *zero point deflection* is expressed by the amount in thousandths of milligrammes by which the unloaded balance differs in successive readings, the second of which has taken place some time after the first. It is considered to be positive if the alteration is from the left to the right and negative in the contrary case.

2. The true weight of the dried material is found by adding the zero point deflection with the reversed sign, that is, a positive zero point deflection must be subtracted from the weight which is found and a negative value must be added.

One may readily demonstrate the effects of air currents inside the balance case by two experiments. If one places a body of temperature higher or lower than that of the surroundings, for

instance a block of copper, near the left pan of the balance, one finds, after a short interval, in the first case a zero point deflection to the left, and, in the other case a similar deflection to the right, and the zero point returns to normal after removing the disturbing object. On touching the side door of the closed balance case with the palm of the hand a zero point deflection is also observed. On then opening the balance front and both side doors the original zero point is restored. It follows that during weighing no objects, such as weight forceps, copper block, and so forth, which are not already there, should be left in the balance case, and, on the other hand, that objects which are always necessarily used when weighing, such as weights, counterpoises, and counterpoise flasks, together with the necessary supply of shot for the latter, should be kept permanently in the balance case, where, moreover, they are best protected from dust. Since generally introducing weighing with a counterpoise in the year 1912 I have used only very few of the weights which are provided with the microchemical balance, namely, the 50 mgm., two 20 mgm. and 10 mgm. weights. The accuracy of these must occasionally be tested, as after use for some years they show a remarkable tendency to become rather heavier. By brushing them, washing them with water and alcohol, and finally polishing them carefully with a cloth, or in extreme cases with rough paper, they are restored to their original exact correspondence with the rider readings. They are best kept in the balance case in a small flat dish, the bottom of which is lined with black velvet. For reasons already referred to, one should not keep the left hand in the neighbourhood of the balance for longer than is absolutely necessary for opening and closing the arrestment, and should make it a rule to leave the balance case open before commencing any series of weighings in order to ensure complete elimination of any possible differences of temperature and moisture between the air within and without the case. We refer to this as " acclimatisation."

In spite of the greatest care and cleanliness it is necessary thoroughly to clean the balance from time to time. This is particularly necessary should it be found that the arresting contacts adhere and cause the balance to be pulled to one side or the other on releasing the arrestment. Uncleanly manipulation when erecting the newly arrived balance may cause this to occur for a considerable time.

The doors are opened, both rider carriers removed and the balance dismantled by removing the pans, the suspensions and

the beam, which may conveniently be placed in consecutive order on one of the rider carriers which is deposited horizontally. The floor plate of the balance is well cleaned with moist gauze, the pans and suspensions are rubbed with chamois leather, which has been washed free from fat and acids and sharply dried, and one then carefully brushes the notched rider scale of the beam and rubs all twelve arresting contacts on the beam, the suspensions and the pillar arrestment energetically with dry chamois leather. In one case of apparently irremovable stickiness of the arresting contacts I finally achieved success by painting the contacts, that is both the pans and the hemispherical arrests, with a paste of previously ignited talc and alcohol, and rubbing off the former with chamois leather after drying. Finally, one cleans the knife edges and the corresponding supports, also with dry chamois leather. This operation is best conducted with the help of a watchmaker's lens, which is indispensable both for mounting the balance and for microanalytical work in general, and should always be in the pocket of the microanalyst. The balance is now re-erected and tested to see whether the zero point has been appreciably altered. This is always the case if one has touched the vane. If the zero point deflection is large, it is approximately corrected by the small screw on the vane. It must be mentioned that handling this latter with the fingers causes an additional deflection of the zero point which lasts for some time, and it is therefore particularly desirable, in correcting small variations, to turn the screw with forceps in order to avoid such warming of the vane.[1] The final adjustment of the zero point, which is concerned with the last 0·01 to 0·02 mgm., is best effected by means of the two levelling screws of the case.

I now wish to mention a circumstance which may influence unfavourably the perfect action of this balance. Very fine hairs may become attached under the central knife edge, under the suspension knife edges, and also to the end of the pointer, causing obstruction to the oscillation and abnormal behaviour of the balance. Such faults are liable not to be detected by the inexperienced if attention is not directed to them.

It is very important for the satisfactory working of the balance that the directions for mounting, which are sent with it, should be read and most carefully followed, as otherwise it is easy for the enthusiastic beginner to cause serious damage.

[1] H. Kuhlmann now provides a light vane with light wings, which renders the zero adjustment particularly easy, instead of the former adjusting screw.

Whilst the zero point of the balance is usually altered on dismounting, as one can hardly avoid touching the vane which adjusts this, the sensitiveness is never changed by these operations if they are conducted with reasonable care, as the two vertically adjusted counter-screws, which control the sensitiveness, are screwed so tightly that their position cannot be changed by ordinary handling, since, at my suggestion, the vane for the zero adjustment is no longer attached between these two counter-screws but independently to the beam. One must, however, distinguish temporary alterations of the sensitiveness, due to warming the vertical screw for some time with the fingers, from permanent alterations which can only be due to serious misuse.

It is advisable to prepare suitable counterpoises for weighing objects which are repeatedly used, such as the boat used in the carbon determination, the micro-platinum crucible, micro-Neubauer crucible, filter tubes and absorption apparatus. Such counterpoises have been used in technical laboratories for a long time. One prepares a counterpoise (Fig. 2*a*) for the platinum boat from thick aluminium wire, which is bent twice so that its three equal, straight portions form the edges of a tetrahedron as in the accompanying illustration. This wire counterpoise is adjusted by filing until, when balanced against the boat, the balance rider is in equilibrium on one of the notches in the neighbourhood of the first milligramme, so that when weighing substances into the boat the rider alone need be used, without additional weights. Similar aluminium counterpoises are also useful for the weighing tubes with holder, which are used for holding the material for the nitrogen determination.

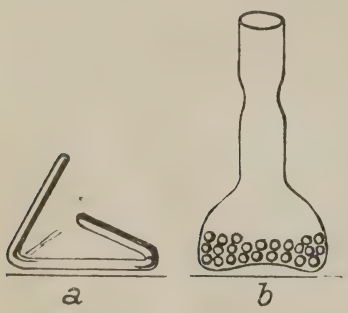

FIG. 2.—*a*. Aluminium counterpoise for the boat. *b*. Counterpoise flask with shot. (Actual size.)

Small counterpoises of fine aluminium wire, weighing about 5 mgm., are very convenient, as they enable one at need quickly to adjust the position of the rider into the neighbourhood of the first milligramme.

For counterpoising heavier objects, such as the others which are mentioned above, I use thin-walled glass flasks of the shape indicated in Fig. 2*b*, which can be obtained from the firm of Kuhlmann in Hamburg, marked with consecutive Roman figures,

so that a special counterpoise flask may be prepared for each object with the help of the necessary amount of small lead shot (Shot No. 15).

It is advisable, in preparing a counterpoise from a flask for one of the above-mentioned heavier objects, first to use coarse shot whilst one places a 50 or 100 mgm. weight together with the flask on the right balance pan. So soon as the balance turns to the left one replaces the weights by one or two fine shot and continues to fill in this shot until the balance again turns to the left. This is the quickest method of effecting the adjustment, and the small amount of work necessitated by preparing these counterpoises is amply repaid during subsequent weighings.

III. The determination of Carbon and Hydrogen in minute amounts of Organic Material

The microanalytical methods for determining carbon and hydrogen in organic substances which were first studied by me in 1910, underwent many alterations and many fundamental changes until the year 1916, in which they attained the final form which has given complete satisfaction during the last few years, as we found that further attempted changes only led to less satisfactory results.

In the process described by me in my first publication in Abderhalden's " Handbuch der Biochemischen Arbeitsmethoden," I was obliged to pass the gases escaping from the absorption train into a mercury gas-holder and then once more through the red-hot combustion tube in order to obtain correct values.

I was subsequently able to avoid a second passage of the gas by increasing the tube filling and using a longer tube. For some time I still used a mercury gas-holder as an aspirator and for controlling the pressure within the absorption train. During this period we already used absorption apparatus which was equal to the highest requirements in respect to constancy of weight, but not in respect to ease and simplicity of manipulation. At this stage of my investigations, Dr. Johann Dubsky, who had studied my methods during a long visit to my Institute in Innsbruck, published a report in the Cöthener Chemiker-Zeitung.[1] At that time we succeeded in reducing the required amount of material to under 2 mgm., which was less than the amount at which I had originally aimed.

[1] Year 38, p. 505.

The experience acquired whilst using the mercury gas-holder established the importance of the pressure and speed relations in the whole system, and especially within the absorption train. Practice was required in order to maintain these relations, and led to the desirability of automatic pressure regulation, which was achieved with the help of a Mariotte flask (1912). The varied experience which I acquired, more especially in conjunction with other colleagues, and at installations in other institutions, led to the detection of a series of sources of error which could not be observed in my apparatus which was in continuous use ; more particularly to the fact that new rubber tubing gave off vapours containing carbon and hydrogen to the gases passing through it until these vapours were exhausted by lapse of time in older tubing. A simple method of artificially maturing the tubing removes these sources of error. Special attention was continuously devoted to the peculiarities of the rubber tubing connections between the various absorption apparatus, and more particularly between the hot end of the combustion tube and the calcium chloride tube, which tended to constitute sources of error ; a solution of the difficulty was finally found by completely impregnating these connecting tubes with vaseline, together with the formulation of, and strict attention to, certain rules for the pressure relations in the whole system of apparatus ; by these means these sources of error could be avoided with certainty. During numerous experiments on the combustion of organic substances containing halogen and sulphur, observations were made on the basis of which a filling for the combustion tube was found, our so-called " universal " filling ; by means of tubes filled with this, all substances could be accurately analysed by combustion, whether or no they contained halogen and sulphur as well as nitrogen. It was found that, in spite of its many objectionable qualities, lead peroxide was an absolutely reliable absorbing agent for higher oxides of nitrogen, although, on the other hand, lead chromate and, more particularly, metallic silver were better suited for retaining halogens and sulphur. For this reason I did not find it advisable to relinquish the use of lead peroxide in spite of the many conveniences which would have resulted.

The field of utility for microanalytical methods was considerably increased in the year 1913 by their application to the analysis of liquids, more particularly in the most difficult determination, namely, that of carbon and hydrogen.

The modifications which the microanalytical determination of

carbon and hydrogen in organic substances underwent were due to the circumstances that each individual condition, and each alteration which was considered, was tested by a long series of blank tests and by analyses of known pure substances. I do not propose to report here on a large number of experimentally tried modifications which were found to be more or less serviceable, but not to be recommended; on the contrary, I have laid most weight on the methods which have proved serviceable under the most severe conditions, and on explaining how and why they have been developed during many years. For this reason, in describing the necessary conditions and apparatus for carrying these out, I will discuss the experience which has been collected. Subsequently I will describe the preparation of the material for analysis and then the actual course of an analysis.

Oxygen, Air and the Delivery Tubing for the same

The oxygen was originally produced from potassium chlorate and manganese dioxide in the usual manner. On controlling all conditions which affect the carbon determination it was found that oxygen obtained in this manner always led to an erroneous increase of weight of the calcium chloride tube and potash apparatus. On the other hand, this phenomenon was not observed when using oxygen obtained from liquid air, and for this reason the latter was afterwards always used for the carbon determination.[1]

Oxygen produced from manganese dioxide and potassium chlorate has been found to be satisfactory only when it is developed very slowly, immediately passed through a series of effective coolers, and then passed, when thoroughly cooled, through the rubber tube into the gas-holder. In the original experiment, therefore, the hot oxygen presumably removed compounds containing carbon and hydrogen from the delivery tube on passing through the same. The recognition of this fact was of exceptional value in improving the microanalytical determination of carbon and hydrogen in organic compounds, and gave me the explanation of a series of phenomena which were then already known but had not yet been explained, such as the fact that a faultlessly erected apparatus in one laboratory, with new tubing of good quality,

[1] *Translator's Note.*—The translator has found that commercial oxygen produced by the fractionation of liquid air may, under certain circumstances, carry minimal amounts of organic matter which seems to be due to the slight vapour tension of the oil used in the compressors, so that, in his opinion, the above statement must be accepted with a certain degree of reservation.—E. F.

constantly gave results which were too high in both analyses and blank tests. This error only vanished after many experiments, that is, after the gases had been passed through the new tubing for a considerable time. I was thus led to submit all india-rubber tubing, which was used in microanalysis to connect the gas-holders with the combustion tube, to a previous artificial ageing process in a drying oven by aspirating air at 100° to 110° C. through it with a water-pump. After such artificial ageing of new tubing it can be used without further trouble for the delivery of pure air and pure oxygen for the determination of carbon and hydrogen. For the above reasons it is convenient and desirable to use lead tubes for longer connections for the above-mentioned gases. Before using these one should distil first alcohol and then water through them, and only use aged rubber connections at points where greater flexibility is required or the use of a pinchcock is unavoidable.

The air used for the analyses also requires a certain amount of attention, for it was found that the use of laboratory air loaded with vapours of organic solvents caused an increase of weight in the blank tests and somewhat high analytical results, as was to be expected. For this reason the air for filling the gas-holder is obtained from the open, for instance, through an open window.

It is obvious that compressed gases from steel bottles can be used directly with the aid of finely adjustable, pressure-reducing valves.

The Pressure Regulator

The early realisation of the fact, in 1912, that for a successful analysis a definite time of contact of the combustible vapours with the individual portions of the tube filling is essential, rendered it necessary to ensure that equal quantities of combustible vapours passed each section of the glowing tube in equal times. This velocity is very greatly dependent on the pressure of the oxygen or air which is introduced. Such gas currents are commonly regulated by the use of pinchcocks on the tubing, and I also used such regulation in the experiments which are described in my first publications, already frequently referred to. With the increasing improvement of the method of analysis, however, it became urgently necessary to adopt safety devices which would render impossible an unexpected increase in the pressure and consequent velocity of the gas stream as a result of careless handling

of the pinchcock. Such control was attained in a very perfect manner by the pressure regulator (*DR.* Fig. 3), which is mounted on a board. This comprises essentially two bell gas-holders, of which one is used for introducing the air and the other for the

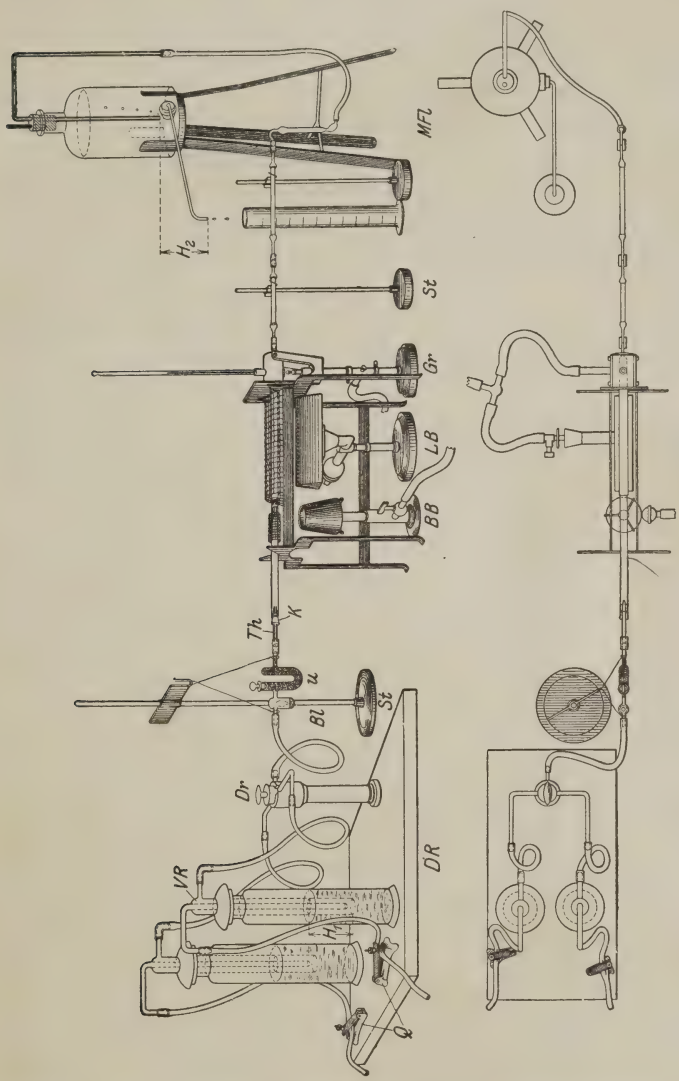

FIG. 3.—General view of apparatus for determination of carbon and hydrogen in elevation and plan. ($\frac{1}{2}$ actual size.) *DR*, Pressure regulator. *VR*, Adjustable tube of regulating gas-holder. H_1, Pressure head. *Q*, Precision pinchcock. *Dr*, Three-way cock. *U*, U-tube with *Bl.* (bubble-counter). *Th*, Tapered thermometer tube. *K*, Rubber stopper. *BB*, Movable burner. *LB*, Tube burner. *Gr*, Heating mortar. *MFl.* Mariotte flask. H_2, Suction head. *St*, Stand.

oxygen. Each consists of a glass bottle about 240 mm. high and of about 60 mm. external diameter, which is about half filled with water containing a little caustic soda ; the mouth of the bottle is closed by a wooden cap. The bell of the gas-holder passes through a central perforation of the wooden cap and is adjustably

held in place by three metallic springs. This bell consists essentially of a glass tube, 20 mm. in diameter and 200 mm. long, within which a narrow glass tube is sealed which reaches to its lower, open end. This inner glass tube constitutes the inlet tube for the gas and is bent twice externally at right angles and connected to the corresponding gas-holder through a matured rubber tube provided with a pinchcock (*Q*, Fig. 3 and Fig. 4).

The gas from the bell gas-holder passes out through a glass tube which is sealed to the side of the bell tube, and which is in turn connected by matured tubing to one limb of the three-way cock *Dr ;* by turning the latter we are able to replace oxygen by air after complete combustion of the material.

On passing air or oxygen into the corresponding pressure regulator the level of the liquid in the bell sinks to its lower rim and any excess of gas bubbles out below, so that the pressure head H_1 (Fig. 3), between the levels in the outer and the inner vessels, is definitely controlled by the height of the inner tube, which is in turn adjusted by the metallic springs, and the pressure head H_1 can, therefore, never be exceeded. As the gas speed in the combustion tube, under the conditions present in the same, is dependent solely on the pressure at which the gas enters, we are able definitely to adjust it by raising or lowering the bell of the pressure regulator, for the lower one places the adjustable tube the higher is the gas pressure and the consequent gas speed, and *vice versâ*. It is obvious that, for reasons of economy, one should reduce the inflow of gas from the main gas-holder into the pressure regulator to such a degree that the necessary pressure in the regulator is just maintained, and that gas bubbles occasionally escape from the lower end of the inner tube at intervals of some three to five seconds. This adjustment is effected by means of the precision pinchcock (Fig. 4).

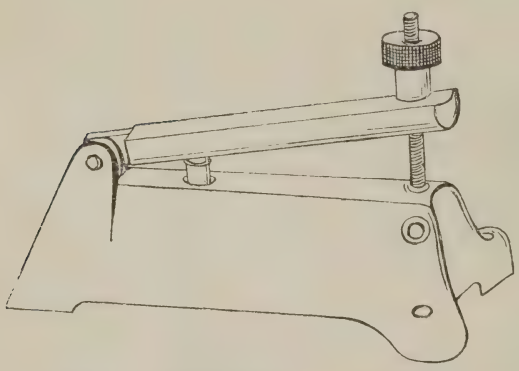

Fig. 4.—Precision pinchcock. (Actual size.)

The third limb of the three-way cock is connected, by means of matured rubber tubing at least 250 mm. long, with the bubble-

counter (Fig. 3 and Fig. 5, *Bl*), which is permanently sealed to the U-tube which serves to dry and purify the gases. The U-tube (Fig. 5) is made from glass tubing of 10 mm. outer diameter, and, as will be seen from the drawing, is closed at one end whilst the other is fitted with a ground glass stopper. This latter limb is connected with the bubble-counter by means of a sealed side tube. The diameter of the inlet aperture into the bubble-counter should not exceed 1 mm.

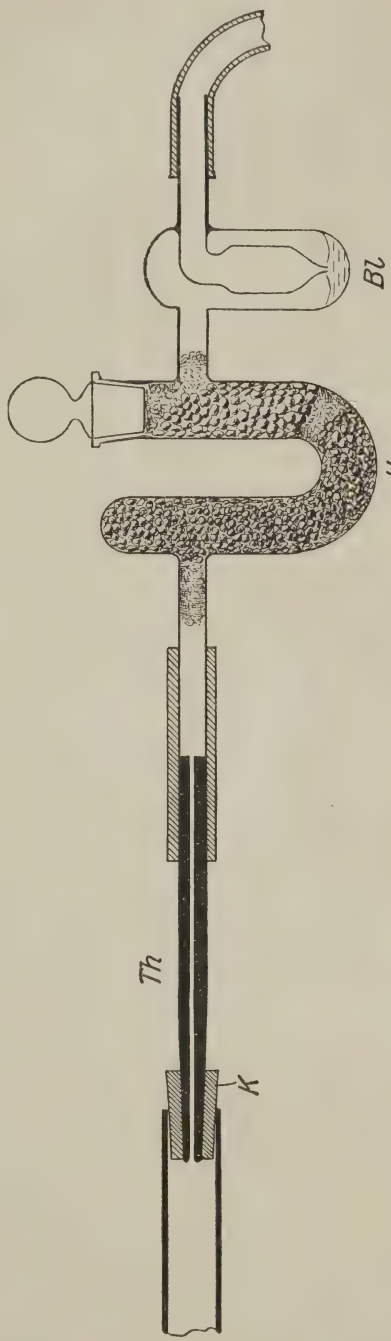

FIG. 5.—The U-tube with the bubble-counter *Bl*, and its connection to the combustion tube by means of the rubber stopper *K* and the tapered thermometer-tube *Th*. (Actual size.)

This apparatus is filled by first inserting a small wad of cotton-wool into the side tube of the sealed limb and then filling in so much granulated calcium chloride through the ground joint of the tube, tapping meanwhile, that about two-thirds of the tube is filled. A small wad of cotton-wool holds this filling in position, and the empty portion of the U-tube up to the level of the side tube leading to the bubble counter is then filled with soda-lime. A wad of cotton-wool is added in such fashion that the soda-lime cannot fall into the bubble-counter. The glass stopper is carefully warmed, covered with Krönig's cement, which is also used

for closing the absorption apparatus (see page 37), and screwed into position. By means of a tapered glass tube, there is now introduced, drop by drop, into the bubble-counter, enough 50 per cent. caustic potash solution, such as is used for nitrogen determinations, to ensure that the tapered lower end of the gas delivery tubes just dips into the liquid. Should an excess be added, this is removed by blowing into the other end of the U-tube. After thoroughly cleaning the side tubes, the apparatus is provided with a suitable wire stirrup, shown in the figure, by means of which it is suspended from the hook of a fairly high stand *St*, and the free end of the connecting tube from the three-way cock is drawn over the side tube of the bubble-counter. The side tube of the closed limb of the U-tube is connected with a tapered thermometer capillary *Th*, 40 to 50 mm. long and of 4 mm. external diameter, by means of a rubber connecting tube which has been treated *in vacuo* with molten vaseline (as are those which are used for connecting the absorption apparatus). The tapered end is inserted into the perforation of the small rubber stopper *K*, which closes the open end of the combustion tube. In order to prevent this rubber stopper from seizing, its bore and its outer conical surface are moistened from time to time as required with a trace of glycerine, the excess of which is removed by careful wiping. Thousands of experiments have shown that this precaution leads to no errors so long as the stopper is treated in the prescribed manner. Originally, large, bulky drying columns, with bubble counters for the inlet gases, were placed between the two pressure regulators and the three-way cock. This arrangement was abandoned at the end of 1913 and replaced by the small U-tube with bubble-counter immediately behind the combustion tube, as I had already arranged it in my original experiments. This change was made because with the former arrangement errors were caused by even the smallest leakage of the three-way cock, and because it was found that one neglected to refill the large drying towers as often as was necessary in order to maintain equalised conditions of the calcium chloride in these and in the absorption apparatus.

Those who have to deal only with readily combustible substances of simple constitution may discard the use of the pressure regulators and connect the gas-holders directly with the three-way cock. This simplified arrangement, which I orginally used and which is described in the publications already referred to, will be found sufficient in a large number of cases in the hands of an expert ;

in the case of substances which are combustible with difficulty, or, more particularly, which contain large amounts of halogen, sulphur, or nitrogen, it has been found by long experience that complete success is only possible with the help of the pressure regulator.

The U-tube and bubble-counter connected with the combustion tube must now be calibrated, that is, the gas volume which passes the tube system in one minute must be correlated with the frequency of the bubbles. This calibration is most readily effected, after connecting the Mariotte flask with the end of the combustion tube, as will be explained later in due course, by measuring the quantity of water which falls into a measuring cylinder during intervals of some two to five minutes. One counts the number of bubbles passing through the bubble-counter during this time. Should, for instance, the quantity of water show that 4 c.c. of gas pass through the system per minute and the corresponding number of bubbles be 12 per 10 seconds, then any gas speed can be determined by counting the number of bubbles per 10 seconds, for a gas speed of 3 c.c. per minute corresponds to a bubble frequency of 9 per 10 seconds, and a gas speed of 5 c.c. corresponds to 15 bubbles in 10 seconds.

Materials for Filling the Combustion Tube for Carbon and Hydrogen Determinations

1. *The Combustion Tube.*—This consists of a tube of hard Jena glass of an outer diameter of 9·5 to 10·5 mm. and a length of at least 400 mm. One end is drawn out to a neck 20 mm. long and of an outer diameter of 3 to 3·5 mm. I have found that it is better to produce this neck, not by drawing out the tube, but by sealing on a tube of the given dimensions made of the same quality of glass. It is important that the bore of the neck should not be too narrow, as otherwise it is easily choked during an analysis by a drop of condensed water. The end of the neck should be cut off vertically to the length of the tube and the edges rounded in the flame without constricting the bore. The same remarks apply to the wide end of the tube.

2. *Fine silver wire, or silver wool,* can be obtained of absolute purity from August Bühne & Co., of Freiburg im Breisgau. One can also now obtain it from P. Haack, in Vienna. It is advisable to reduce the silver wire before use in a current of hydrogen in a glass tube and afterwards to ignite it in a current of oxygen. The same procedure is used for the silver fillings of used tubes, which

are loaded with halogen and sulphur, in order to regenerate them for further use.

3. *Commercial purified Gooch crucible asbestos* is used for the asbestos wads. One should never neglect to ignite it strongly on the lid of a platinum crucible with frequent turning before use.

4. *Lead Peroxide.*—Although the firm of E. Merck, in Darmstadt, was good enough to agree to prepare granulated lead peroxide of groat size according to my instructions, I will describe the process carried out by me. Powdered commercial lead peroxide is heated in an evaporating dish on the water bath with concentrated nitric acid, then digested with water and, after standing for some time, is rapidly washed by decantation with distilled water until the acid reaction has vanished. The slimy residue is then slowly evaporated and, before it is completely dry, is cut into small cubes with a sharp spatula. These are rotated in a roomy wide-necked bottle, either by hand or, more conveniently, in a slowly rotating lathe. In this way the small cubes polish one another and the globules which are obtained can be separated from the powdered material by sieving. The powdered portion can be used for the production of a further quantity of the granulated product after re-moistening.

The use of any adhesive in order to facilitate the granulating process is absolutely debarred. Preparations produced with these led to a waste of much time and trouble until it was found, by comparison with pure preparations, that the higher values for carbon, which were regularly obtained, were due to the presence of the adhesive. Such preparations may be recognised by the greater hardness and darker appearance of the grains. It has also been found very advantageous to place the lead peroxide under a glass bell for an hour in the presence of ammonia before introducing it into the tube, as in this way the duration of the ignition, which is subsequently necessary, is reduced to a few hours, on account of the neutralisation of the traces of nitric acid which are obstinately retained.

The above was the result of my experiences until 1921, at which time I found that the grain size of the lead peroxide was of considerable importance, as with increase of the grain size the difficulty of completely expelling the water and the necessary time of ignition of a newly filled tube were considerably increased. This led me to carry the subdivision of the lead peroxide much further than had already been practised by Kopfer

(*Zeitschrift fur analytische Chemie*, vol. 17, 1872, p. 32), by distributing the powdered lead peroxide on asbestos. I now proceed by stirring Gooch crucible asbestos, which has previously been purified by ignition and by extraction with boiling nitric acid, into the lead peroxide paste obtained by decantation according to the method already described. I use such quantities that the total volume of the moist mass is increased two or three times. By evaporating to complete dryness, and subsequently teasing with two mounted needles, one obtains a loose mass which cannot be separated into its constituents by shaking in a narrow tube in spite of the great difference in their densities. Such a preparation possesses the advantages that a tube with which it has been freshly filled, is ready for use after ignition for one to two hours, and also that, even in the case of substances containing much hydrogen, the passage of 100 c.c. of air after completion of the combustion is quite sufficient to drive the water quantitatively into the calcium chloride tube. The result is also much less dependent on the temperature than when granulated lead peroxide is used. It may at once be mentioned that when using lead peroxide asbestos the tube is filled in a slightly different manner.

5. As the actual oxidising filling of the tube a mixture is used of about equal parts of wire-form copper oxide and lead chromate of groat to hempseed size. It should be thoroughly ignited before filling.

I have latterly found it preferable to prepare the lead chromate by precipitating a dilute solution of lead acetate with potassium dichromate, washing thoroughly by decantation, draining on the filter pump and drying. The powdered lead chromate so obtained is strewn with stirring on to wire-form copper oxide at a bright red heat. Large quantities are necessary, and one continues until the individual grains are impregnated with the molten lead chromate. In this way one obtains a better distribution and greater surface of lead chromate, and also ensures that the wall of the combustion tube is less attacked than when lead chromate is used in lumps.

Filling the Combustion Tube for the Carbon and Hydrogen Determination

It is advisable first to wash the tube repeatedly with sulphuric-chromic acid mixture and then with water, and then to rinse it with distilled water and alcohol and dry it on the pump with gentle warming.

One then pushes a little wad of silver wool up to the neck of the tube to form a layer about 10 mm. long, using a clean glass rod of about 4 mm. diameter with sharp edges. One then introduces a very small wad of freshly ignited Gooch crucible asbestos and presses it against the silver wad with the glass rod. Its use is to protect the latter from contamination with lead peroxide dust. An asbestos layer of 2 mm. thickness is quite sufficient for the purpose. Granulated lead peroxide is then added for a length of 20 to 25 mm. One then finds the whole inner surface of the combustion tube to be coated with lead peroxide dust. It is advisable to remove this by wiping it with a plug of cotton-wool attached to the end of a wire. One then adds freshly ignited asbestos in three portions, pressing it home with a glass rod each time. In this manner a choking plug is formed about 7 mm. long, the use of which is to produce the greatest gaseous friction of the whole system at this point, as a consequence of which equal volumes of gas pass the plug in equal times. It is now advisable to test the degree of gaseous friction, that is, the density of the plug, and for this purpose all that is necessary before proceeding further with the filling of the tube is to attach to it the U-tube with the bubble-counter and to determine whether, at a head of about 50 to 70 mm. of water in the pressure regulator, about 3 to 5 c.c. of gas pass through the tube per minute. If one is using a bubble-counter which has already been calibrated it is sufficient to determine whether the necessary bubble frequency, which is already known, is attained under these conditions. A loosely packed layer of silver wool, about 30 mm. long, is then placed on the choking plug and is followed by a thin wad of asbestos which is not too tightly compressed. There follows a layer, about 140 mm. long, of the mixture of copper oxide and lead chromate, after which the empty portion of the tube is once more cleaned with a wad of cotton wool and the filling kept in place with a small wad of asbestos. Beyond this there follows a further layer of silver wool, 25 to 30 mm. in length. When lead peroxide asbestos is used it is necessary to alter the tube filling slightly in order to prevent this loose material from being strongly compressed, which would destroy its good qualities. For this reason I first insert the choking plug in the manner already described, immediately behind the neck, and follow on with the lead peroxide asbestos for 20 to 25 mm. by allowing this to slide into the tube and tapping lightly. After wiping out the walls of the tube, I then add a small asbestos wad, then a layer of silver about 30 mm. long, and then

once more an asbestos wad and the further tube filling as already described.

To prepare the tube for a combustion it is wrapped in the neighbourhood of the second layer of silver with a strip of asbestos paper by means of which it is held in the heating mortar. This strip of asbestos paper also prevents the production of an air current through the mortar which would cause temperature changes. That portion of the tube which projects beyond the heating mortar is placed on the combustion frame in such a manner that it lies on the two end plates of the latter, and that the heating mortar is separated from the neighbouring wall of the combustion stand by not more than 1 cm. By this arrangement a portion of the second layer of silver lies within the combustion stand and within that portion of the tube which is heated by the tube burner. A roll of iron gauze 180 mm. long is wrapped loosely round the filled portion of the tube within the combustion stand and a similar roll of iron gauze 40 mm. long is wrapped round the empty portion of the tube.

A slightly conical rubber stopper, the outer surface of which is moistened between the fingers with a minute quantity of glycerine, is then inserted into the wide open end of the tube and receives the tapered capillary *Th*, of Figs. 3 and 5. Connection is thus established by way of the U-tube and bubble-counter with the pressure regulators and gas-holders. The tube is then ignited for some hours, during which very slow currents of oxygen and air are alternately passed through it. It is then advisable to carry out a blank experiment in order to be certain that all the conditions for a reliable analysis have been secured.

A perfectly filled and ignited combustion tube, if carefully treated, can be used for 200 to 300 analyses if it is not unduly heated, and if the layer of lead peroxide is never exposed to a higher temperature than that of boiling cymene, which should actually be impossible. It is only when a consecutive series of combustions of substances containing halogen and sulphur is carried out that the last portion of the silver filling adjacent to the boat is considerably affected. In such cases, therefore, it is better to remove this after about ten to twelve such analyses and replace it by new silver wool.

The method of filling the combustion tube which has just been described, which may at first sight appear rather complicated, is the result, on the one hand, of considerable thought, and, on the other, of a whole series of experiments.

It is obvious that in the case of substances containing only carbon, hydrogen and oxygen, only an oxidising tube filling is needed. It is well known that suitable materials for this purpose are, more particularly, copper oxide, also lead chromate, and, finally, platinum contacts, which were first generally used by Kopfer for this purpose in elementary analysis. If, however, the substance to be analysed contains nitrogen, two methods are available for fixing the higher oxides of nitrogen, which would otherwise cause errors through excessive increase of weight in the soda-lime tube, namely, either red-hot metallic copper or lead peroxide. It must be emphasised here that the use of red-hot copper in the microanalytical determination of carbon and hydrogen in organic materials by combustion in a stream of oxygen meets with still greater difficulties than does its use in macro-analysis, as the excess of oxygen which is used would, although small, suffice to oxidise the surface of the copper. For this reason lead peroxide only remained as a generally applicable reagent for the reliable absorption of the nitrogen oxides, for metallic silver is quite unsuitable for this purpose, as was already shown experimentally by Emich in the year 1892.[1] This fact is not inconsistent with the statements of various investigators who have obtained accurate results in the analysis of nitrogenous substances with the use of a silver coil, in so far as these substances were amino-, imino- or similar compounds, for these substances yield on combustion elementary nitrogen only, or, at most, possibly nitrous oxide, but not the higher nitrogen oxides. On the other hand, the combustion of nitro- and probably also of nitroso- and oxyazo-compounds leads to the inevitable production of higher nitrogen oxides, and, therefore, such compounds only should be used in the investigation of this question. If the organic material also contains halogen and sulphur, lead peroxide, which is recommended and used for the purpose of retaining these, is not always sufficient with the gas velocity which has been found by me to be otherwise suitable and sufficient. This is particularly the case when the lead peroxide already contains a little nitrate. The best reagent for the absorption of halogen is heated metallic silver, and lead chromate has been found particularly suitable for retaining the oxides of sulphur. By placing these two reagents in front of the lead peroxide the burden on the latter is lightened and its only function, that of absorbing the higher nitrogen oxides, remains unimpaired. The possibility of removing from time to time that

[1] *Monatshefte für Chemie* (1892), 13, pp. 79–83.

portion of the silver filling which is most affected enables the remainder of the tube filling to be permanently maintained in a pure and effective condition.

I owe to the chief analyst of the Badische Anilin- und Soda-Fabrik, Dr. Birkenbach, a substance of properties which render it extremely suitable for testing various tube fillings, namely, 1-3-4-trichloro-dinitro-benzene, of which he supplied me with several grammes from the collection in the main laboratory of his firm. With this preparation I also tested the tube filling recommended by Dr. Dubsky.[1] I only varied his directions in so far as, in addition to the final silver filling, I placed a second filling immediately in front of the boat, and that I used a mixture of copper oxide with lead chromate instead of copper oxide alone. Also, for reasons already mentioned, an asbestos plug was inserted between the first silver filling and the oxidising layer. Of course, in order to maintain the most favourable conditions, the pressure regulator was so adjusted that a gas stream of 3 c.c. per minute was used. Although the Mariotte flask was not used all the values found were too high :

Found.		Calculated for $C_6HCl_3N_2O_4$.
1·30 per cent. H.	1·40 per cent. H.	0·37 per cent. H.
29·79 per cent. C.	29·93 per cent. C.	26·53 per cent. C.

Dr. Dubsky was kind enough to analyse the same preparation, which was shown by the melting point to have come to hand in a pure condition, without lead peroxide but with the silver coil in front as an oxidising filling. He naturally used neither a choking plug nor a pressure regulator. His figures were :

Found.			Calculated for $C_6HCl_3N_2O_4$.
1·17 per cent. H.	1·66 per cent. H.	1·38 per cent. H.	0·37 per cent H.
28·54 per cent. C.	29·86 per cent. C.	30·68 per cent. C.	26·53 per cent. C.

Thus the errors in the analytical results of Dr. Dubsky show greater deviations from the theoretical values than my own. This fact is no doubt simply explained by the fact that the exact regulation of the gas stream by the choking plug and the pressure regulator represents a refinement of the analysis which cannot be dispensed with, more particularly in the case of substances containing halogen and sulphur.

These results also confirm Emich's observation that heated silver is incapable of decomposing the higher nitrogen oxides. The contrary view, which is erroneous, appears, unfortunately, to

[1] *Cöthener Chemiker-Zeitung*, March 4th, 1916.

be widespread, as is also a prejudice against the use of lead peroxide as a reagent in elementary organic analysis. Finally, I wish to remark that with my "Universal filling," and other arrangements, trichloro-dinitro-benzene has been subjected to combustion without any difficulty and with correct results in my laboratory, not only by myself but by most of those who have tried the experiment. I do not wish these remarks to be interpreted in the sense that the proposed simplified tube filling of Dr. Dubsky is unsuitable under all circumstances. On the contrary, I was able to assure myself in Innsbruck that with such a tube filling, more particularly when lead chromate is used, correct analytical results can be obtained with all substances in which the nitrogen gives rise to no higher nitrogen oxides on combustion, and that it can also be applied to substances containing halogen and sulphur and correct values obtained, if the choking plug, which Dr. Dubsky has unfortunately discarded, is used, and the necessary time of contact of all the gas particles with the heated tube filling is thus assured.

Dubsky himself was ultimately forced to accept this view by the experiments which I carried out,[1] and have just described, and then chose once more to introduce the use of lead peroxide as "a sort of novelty," as the matter was expressed by A. Schöller. Schöller, who worked quite independently and has no connection with me and my experiments, expressed himself on the various modifications, which have been proposed from time to time, as follows in the *Zeitschrift für angewandte Chemie*, Year 34, November 22nd, 1891, No. 93, p. 582: "As is emphasised by Pregl, reliable results are only obtained with quantities below 5 mgm., especially in the case of materials which are burnt with difficulty. The beginner too easily makes the mistake of trying to render the work easier by using larger quantities. If the weighings are carefully carried out, and the necessary absolute cleanliness is observed, the errors are introduced, not by the weighings, but mainly by incomplete combustion, and this possibility is increased when larger quantities of material are used owing to the narrow bore of the tube. It is also necessary to ensure a sufficient degree of dryness of the calcium chloride and the presence of sufficient moisture in the soda-lime. The soda-lime tube should be filled before each series of combustions, and each filling is sufficient for at least six analyses. The calcium chloride tube suffices for about twenty analyses, and when it is refilled the drying tube should also

[1] *Berichte* (1917), vol. 50, p. 1711.

be refilled. For exact work about 2 mgm. of material suffices for useful results.

"The so-called simplified or semi-microanalysis of J. V. Dubsky consists essentially in increasing the amount of material to 5 to 15 mgm. and discarding the choking plug, the pressure regulator and the Mariotte flask. At first the lead peroxide and the heating mortar were also discarded, but were then re-introduced as a sort of novelty. Whether the increased quantity of material is advantageous appears to me very questionable on the above grounds. It is certainly a mistake to discard the choking plug and the pressure regulator, as these do not render the work more difficult, but, on the other hand, facilitate it considerably. When the choking plug is used it is quite impossible that air can be sucked into the tube from without, as the pressure within is equal to about 100 mm. of water ; yet this possibility and its prevention are carefully considered by Dubsky. Should the apparatus not be perfectly tight at this point there is merely a slight escape of oxygen when using a choking plug, for the reasons already explained, and, therefore, a rubber stopper can never give rise to errors if it is not overheated or rubbed with too much glycerine, and a ground joint is consequently quite unnecessary. Should the vapours of the material travel back to the stopper the analysis is spoilt in any case. This possibility is, however, most securely guarded against by the pressure regulator, as when this is used any sudden increase in the quantity of vapour evolved is at once indicated by a diminution in the number of bubbles. I do not wish to deny that useful results can be obtained by Dubsky's methods, but am, at the same time, very doubtful whether these conduce to simpler and easier working than the established methods of Pregl or their rational modification by the use of absorption apparatus with ground-glass joints."

The Heating Mortar and the Combustion Stand

As has already been explained, lead peroxide is such an excellent absorption agent for the higher oxides of nitrogen that it should always be used, although many precautions are necessary in the treatment and use of this reagent, particularly if sulphur and halogens are present together with nitrogen, whatever may be the form in which this latter element is present in the substance to be analysed.

Lead peroxide has the property of obstinately retaining water, the amount of which decreases as the temperature rises, but for

any given temperature the amount of water which is retained is constant. In order, therefore, to obtain correct hydrogen results, it is necessary to maintain the lead peroxide at a constant temperature not only during the combustion, but also when preheating the tube. This object was at first attained by me by means of a solid cylindrical copper block through a central perforation in which the combustion tube was passed. On account of its shape I called this the copper "mortar." Its temperature was measured by a thermometer let into the top, was regulated from below by a non-luminous micro-burner, and usually maintained at 180° C. In order to regulate the temperature in a simpler and more certain manner the mechanic at the Innsbruck Physiological Institute, Franz X. Eigner, built for me a hollow mortar *Gr* (Fig. 6), in which a liquid of high boiling point was constantly maintained at the boil. Originally I used for this purpose the petroleum fraction which boils between 190° and 220° C. Later, at the suggestion of my colleague, Fritz Strauss, of Strassburg, I used technical cymene, by which means a constant temperature of 176° C. can be maintained, and which also offers certain other advantages over petroleum. This hollow mortar is a hard-soldered hollow cylinder, 65 mm. long and of 30 mm. external diameter, which surrounds a central cylindrical chamber of 11 mm. bore to hold the combustion tube. At the top a glass air condenser *St* is fixed by means of a screw fitting, to which it is cemented, and the apparatus is heated from below by a non-luminous micro-burner. In addition, there is a cylindrical pocket of 3 mm. bore throughout the whole length of the hollow mortar below, by means of which the copper heater *KB* is inserted, which serves to warm the inlet of the calcium chloride tube in the vicinity of the two capillary constrictions and which can be adjusted to warm various portions of this region. The further arrangement of the apparatus will be clear from Figs. 3 and 6.

I have latterly carried out successful experiments with massive heating mortars of aluminium. I was led to try these by the favourable results, already mentioned, which were obtained with lead peroxide asbestos. The hollow mortar is, however, doubtless the more perfect instrument, as it relieves the analyst of all trouble for hours on end.

On account of the high price of cymene I have latterly reverted to the use of petroleum, but only after carefully purifying the latter by shaking ordinary lamp oil three to five times with one-fifth its volume of concentrated sulphuric acid in a separating

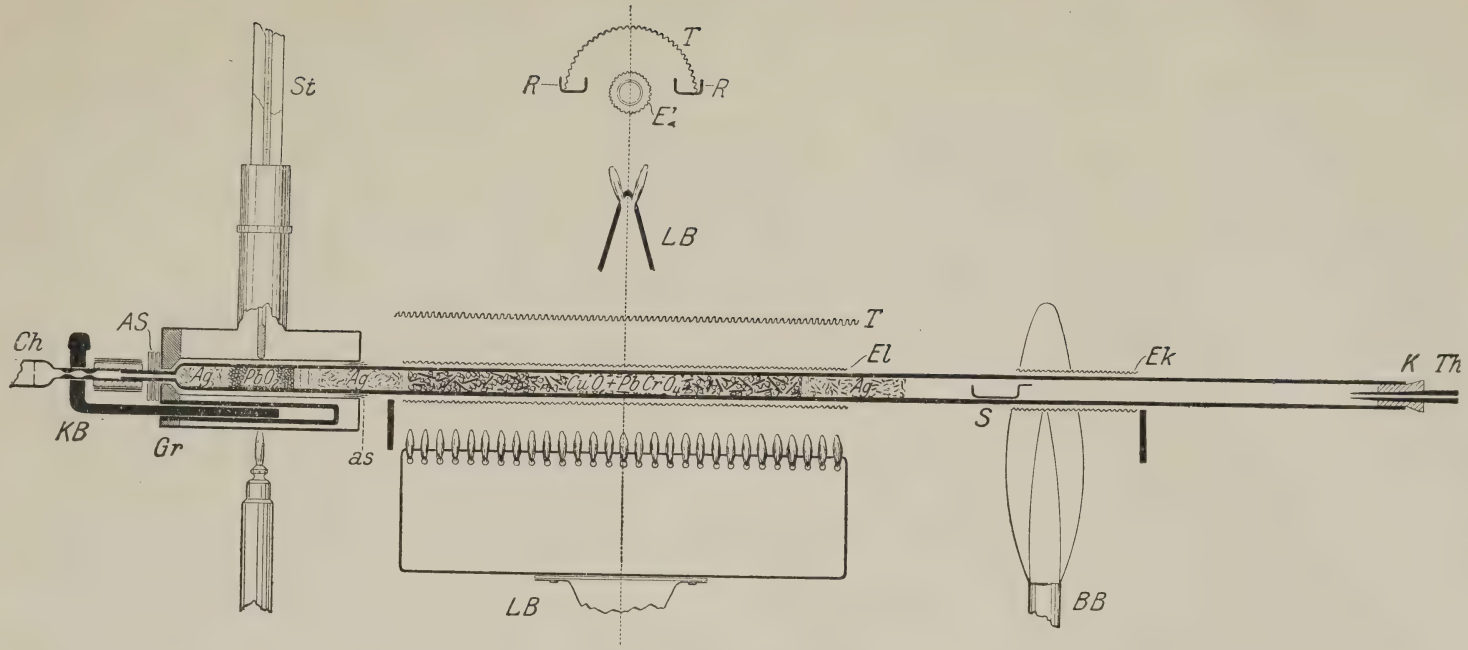

FIG. 6.—The filled combustion tube in position for analysis. ($\frac{1}{3}$ actual size.)

Ch, Calcium chloride tube. *KB*, Copper heater. *Gr*, Hollow mortar. *St*, Condenser tube. *LB*, Tube burner. *BB*, Movable burner. *T*, Wire gauze tunnel. *El*, Long roll of wire gauze. *Ek*, Short roll of wire gauze. *R*, Runnels of sheet iron. *as*, Asbestos wrapping. *AS*, Asbestos disc.

funnel and afterwards washing it thoroughly with water and caustic soda solution. After drying the liquid so obtained with calcium chloride one obtains, by fractional distillation, a portion boiling from 170° to 200° C. which is suitable for filling the mortar, and which remains practically colourless after using for several weeks. My former assistant, Dr. Zima, at present employed by Merck, in Darmstadt, uses phenol for the same purpose, and closes the top of the condenser with a calcium chloride tube to prevent the entrance of moisture.

The constant temperature which is absolutely necessary for correct determination of the hydrogen is assured in an entirely satisfactory manner by the hollow mortar. If the neck of the combustion tube, which projects at one end of the mortar, is wrapped with a strip of asbestos paper, 10 mm. wide at the other end, so that a reasonably tight joint is there made, this prevents secondary temperature variations caused by circulation of air. A few asbestos discs, with central perforation *AS*, are also placed over the neck of the tube in order to prevent excessive heating of the neighbouring rubber connection.

After fixing the filled combustion tube with its asbestos wrapping into the hollow mortar in the manner just described, it is possible, by altering the various adjustments, so to arrange the projecting portion of the tube that it fits horizontally without strain into the rectangular notches at both ends of the combustion stand. The distance between the hollow mortar and the neighbouring end of the combustion stand should amount at most to 10 mm., as shown in Fig. 6.

The combustion stand rests on four legs, is 300 mm. long, and is fitted at both ends with the two above-mentioned rectangular notches which carry the combustion tube, and at the sides with two narrow metal gutters *R*, parallel to the combustion tube, which serve to carry a coarse piece of iron gauze, bent to a U-shaped section, to cover the filled portion of the tube and form a tunnel-shaped space about 180 mm. long, which is referred to in the figure as gauze-tunnel *T*. This arrangement has always been found the best to ensure the uniform heating of the filled portion of the tube, and we have hitherto seen no reason to modify it. A similar movable wire gauze tunnel, 50 mm. long, placed above the movable burner is very useful in the case of substances which burn with difficulty.

The filled portion of the tube which is to be heated, is protected from direct contact with the flames of the tube burner,

and also against distortion, by covering it with a thin roll of fine iron gauze *El*, about 190 mm. long.

Originally I occasionally used a sheet iron support of rectangular section, about 20 mm. wide, for the combustion tube ; both ends of this were slotted and bent in such a way that the projecting tongues fitted into the right-angled slots at the ends of the combustion stand, and that the lateral projections fitted into the lateral gutters. This carrier, which was 180 mm. long, was lined with damp asbestos paper which served, after drying, as a support for the heated portion of the combustion tube.

The tube is heated by means of the tube burner *LB*, the nature of which is sufficiently clear from diagrams 3 and 6. This apparatus was also constructed to my specifications by the University mechanic, Franz X. Eigner, in Innsbruck, and enables the height of the flames to be regulated with exceptional precision whilst maintaining them approximately uniform.

The apparatus for the determination of carbon and hydrogen can be set up on any working bench. The best protection of the bench top from heat is afforded by a sheet of uralite. A special room separated from the ordinary working laboratories is desirable for this determination. The balance room is quite suitable if the apparatus is placed near the wall opposite to the balance. It should not be placed close to the balance, because the heating would cause constant variations in the zero point ; it is undesirable to carry out the combustion in places which are too far from the balance room, as the absorption apparatus is then subjected to unduly high temperature differences on its removal.

Naturally, attempts have been made to heat the tube electrically. Personally I have no experience in this direction.

The Absorption Apparatus

The experiments mentioned on pp. 1322 and 1323 of my former publication showed that the application of ante-chambers between capillary constrictions in front of the absorbing agents for water and carbon dioxide ensured a high degree of constancy of weight. This was found to be due to the fact that the diffusing moisture traversed a region of wide cross-section between the two capillaries resulting in a variable diffusion potential. For this reason, in 1912, I arranged this apparatus in a more convenient and handy form by separating the actual absorption chambers from the two ante-chambers by diaphragms with a central capillary opening. The apparatus used by me at that time was also provided with

two capillary constrictions on each connecting tube, between which a slight widening of the bore existed. The absorption chamber of the calcium chloride tube, which was rather smaller than the other, contained calcium chloride only, held between cotton-wool wads. The potash apparatus, which was somewhat longer, contained at one end of the absorption chamber a 30 mm. layer of calcium chloride of groat size, held between plugs of cotton-wool and separated by an empty space about 50 mm. in length from a filling of loose glass wool extending to the other end, as is shown in the appended drawing (Fig. 7). The glass wool of this potash apparatus was treated after every two combustions by sucking up 50 per cent. caustic potash lye into it, after which this was blown and jerked out. It was then necessary to rinse out the moistened ante-chamber by repeatedly drawing water into it, and afterwards to dry it, which many found difficult, and which was in any case tiresome and inconvenient. A further inconvenience of this absorption apparatus consisted in the fact that the calcium chloride which it contained became overloaded with water after some time, and it became necessary to remove the absorbed water under reduced pressure at high temperature, for which purpose the so-called "regenerating block" was found very useful.

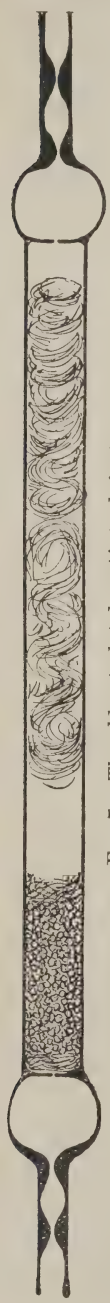

Fig. 7.—The old potash tube. (Actual size.)

Although this apparatus fulfilled the highest requirements with regard to constancy of weight, the circumstance that its treatment required considerable attention gave rise to further modifications.

A modification leading to the present form of absorption apparatus contained slightly moistened soda-lime in place of the glass wool and was equally satisfactory in use to the one just described, but after being used for four or, at most, five times it had to be cut open, refilled and sealed up carefully before the blowpipe.

After learning how to grind glass as well as blow it, I was able, in October, 1913, to produce absorption apparatus which can be refilled by opening and closing a ground-glass joint. The tubular absorption apparatus was furnished at

one end as before with an ante-chamber 10 to 12 mm. long, and a connecting tube with two capillary constrictions of 0·1 to 0·2 mm. diameter, and at its open end with a ground hollow stopper 10 to 12 mm. long. The interior of this stopper communicates with the main chamber of the apparatus through a fine perforation of 0·2 to 0·3 mm. diameter, and is also provided with a connecting tube containing two capillary constrictions. The tubular portion of the apparatus has usually an external diameter of 8 or, at most, 10 mm., and the length of the absorption chamber is 80 mm. (Fig. 8). Obviously this type of apparatus can be used either as a calcium chloride tube or as a soda-lime tube. The soda-lime tubes used by me are usually larger and have an absorption chamber 120 mm. long, as it is then possible to use them for a large number of analyses, at least four, without changing the filling.

Fig. 8.—New absorption apparatus with ground joint. (Actual size.)

Constrictions in the connecting tubes, which are too wide, can readily be made narrower by softening them in the flame of a micro-burner. Very narrow portions, which are very inconvenient, particularly if the side tube is inclined downwards, can be blown out when softened by carefully blowing down an attached piece of rubber tubing, the apparatus being meanwhile closed with a rubber cap.

Originally I made this apparatus wider at the middle than at the ends in order to increase the possible soda-lime filling, but have relinquished this and prefer cylindrical tubes, as with these latter the time of cooling necessary to attain constant weight is never longer than fifteen minutes.

The ground-glass joint is made good by the use, when slightly warmed, of Krönig glass cement, which is made by melting together one part of white wax and four parts of colophonium (resin). The excess of cement is removed after cooling, first mechanically, and then by wiping with a rag moistened with benzene. If the apparatus is to be used for the absorption of water the ground glass joint must obviously not adjoin the warmed neck of the combustion tube. It is filled as follows :

In order to prevent the escape of calcium chloride dust one

first introduces a small plug of cotton-wool. After this one introduces either two or three large pieces of porous calcium chloride, or, if one has only the commercial preparation of groat size to hand, one introduces side by side with this, over a distance of 10 to 15 mm., a plug of cotton-wool. One then fills up with porous calcium chloride of groat size to below the ground joint, covers with much cotton-wool and cements the joint. By using either large lumps of calcium chloride, or a cotton-wool plug side by side with smaller lumps of that material at the point where the combustion gases enter, a sudden stoppage of the tube by liquefaction of the calcium chloride is avoided, even when the apparatus has been used for a long time.

The apparatus is then filled with carbon dioxide in the direction in which the gases will ultimately pass through it, that is, from the side tube without ground joint to the other end, and is allowed to stand for ten minutes under pressure when filled with this gas. This is most conveniently achieved by attaching the tube to the combustion tube, to be described later, which is used for the nitrogen determinations and is always kept filled with carbon dioxide under pressure. The carbon dioxide is then removed by passing a stream of air in the same direction. The Mariotte flask is best used as an aspirator, as the quantity which is aspirated, and which should be about 100 c.c., can readily be determined by the water which flows away. The absorption apparatus filled in this way, when used continuously and daily, can be used at least fifty times, until it increases in weight by about 100 mgm.

For the absorption of carbon dioxide the apparatus is first filled with a layer about 5 mm. long of firmly compressed cotton-wool, after which one introduces a layer of 30 mm. of porous calcium chloride of groat size and then a small cotton-wool plug to separate this from the following material. This consists of soda-lime of groat size, of which a portion is removed from the stock bottle before use into a fairly large evaporating dish, in which it is slightly moistened when in a thin layer with a spray of water, and then kept in a well-closed bottle. As is well known, this moistening is essential for complete absorption of carbon dioxide, but it should not be sufficient to cause the individual grains to cake together. In this case also the apparatus is filled to the bottom of the ground joint, the filling covered with a small moistened plug of glass wool and the joint cemented.

100 c.c. of air is now aspirated through the calcium chloride tube by attaching the Mariotte flask exactly as in the case of the

soda-lime tube. During this operation one should add, if necessary, as will later be explained, a small drop of water inside the stopper which is used as an ante-chamber, warming the side tube meanwhile.

The filled absorption tubes are closed by suitable caps of rubber tubing and placed on a metal penholder stand, which supports them at two points only, near the balance.

These tubes, and also those previously described, show various peculiarities which need to be discussed. One must make it a

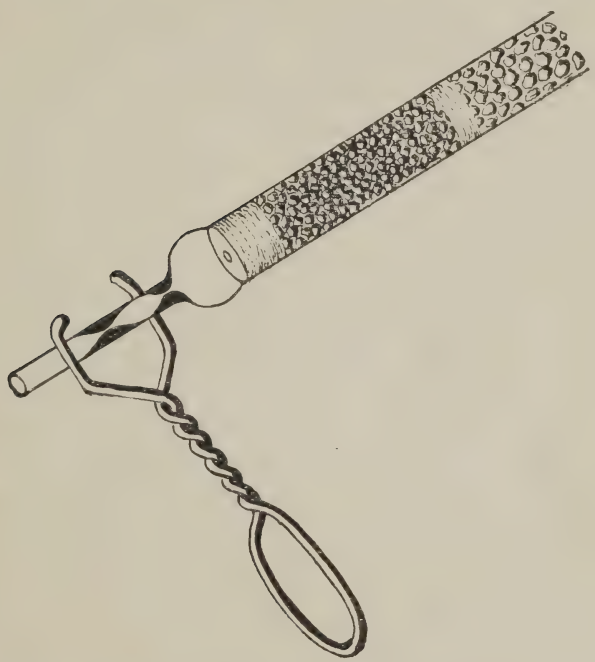

Fig. 9.—Fork for placing the absorption tubes on the balance. (Actual size.)

rule to clean them before each weighing by wiping them carefully all over with a piece of moist flannel and then with two pieces of chamois leather until one feels that these slip over them easily. The movement during these operations should be partly rotatory and partly from the middle towards the ends, but never in the other direction. The fact must be emphasised here that with practice one is able to detect during this operation the minutest traces of impurities, often weighing a few hundredths of a milligramme only, but we must also emphasise that immoderately strong friction may produce an error to which we will refer later. For this reason it is only permissible to clean the apparatus energetically

before the beginning of the analysis, as afterwards the absorption tube may only be removed with freshly washed hands, and unnecessary handling is generally to be avoided at this stage. It suffices after completion of the combustion to wipe them all over, but quickly and quite gently.

After handling and wiping each tube suffers apparent loss of weight, which is presumably mainly due to the warming of its surface. The consequence is that, immediately after wiping, an increase of weight is observed which is considerable during the first five minutes, namely, 0·1 mgm., or even a multiple of this value. Between the fifth and tenth minute it is only a few hundredths of a milligramme, and at the fifteenth minute a value is reached which remains unchanged even for a further half-hour, if all the necessary conditions are observed in the weighing room and with regard to the apparatus. After wiping the tubes must no longer be handled. They are removed and placed on the balance by means of aluminium forks (Fig. 9).

The above is the typical behaviour of the absorption tubes after wiping. Very occasionally it has been observed that the tubes show a maximum weight immediately after wiping and then become steadily lighter. I investigated this phenomenon, together with Professor Karl Brunner, of Innsbruck, and we came to the conclusion that it is due to excessive dryness of the chamois leather used for wiping the apparatus, which causes electrostatic charges on the glass surface of the tube ; it can be completely avoided by keeping the pieces of chamois leather in a glass bottle into which a moist flannel rag is occasionally introduced for an hour.

Apart from the constancy of weight, which a tube acquires in ten to fifteen minutes after being properly treated as described, it must also have the property, after being handled as in practice or in a blank experiment, of showing the same weight after wiping once more and allowing to stand for the regulation time. This property is only shown when the room in which the weighings are conducted has a temperature equal to, or higher than, that in which the combustion takes place. If a tube at a higher temperature is brought to a very cold balance-room, the contraction of the air inside the tube during cooling suffices to draw a weighable quantity of water vapour into the tube and into contact with the absorption agents, and one then finds positive errors of weight up to 0·03 to 0·04 mgm., or even more. In the case of a calcium chloride tube, this error is not very serious, as the amount of

hydrogen corresponds to one-ninth only of the weight increase. The case is otherwise with the soda-lime tube. This error is therefore guarded against by placing a doubly folded piece of moistened flannel over the tube in order to cool it during the whole course of the combustion. The same error, due to the entrance of moist air during cooling of the tube, is also produced if the wiping is so energetic that the tube is warmed sufficiently to cause this to occur. On sultry summer days one may observe that, in spite of precautions, the soda-lime tube shows noticeable increases of weight of about 0·02 mgm. and sometimes rather more per hour. This phenomenon can be completely prevented by introducing a small drop of water into the tube, through the constricted channels, into the ante-chamber of the stopper whilst aspirating air through it, meanwhile warming the two capillaries slightly in order to drive the last traces of moisture into the tube. Tubes prepared in this way show a perfectly constant weight even on sultry summer days, for the small drop of water inside the stopper causes about the same degree of evaporation outwards as of diffusion of water vapour inwards at the other end.

One other effect must be mentioned here. For some time we noticed that the absorption tubes, after being finally cooled on the stand outside the balance case, regularly lost weight by 0·01 to 0·02 mgm. on the balance and then only attained a constant weight. The explanation was that the tubes, when cooled directly over a marble slab, were always colder than the inside of the balance. The effect has disappeared now that we no longer place the stand holding the absorption tubes directly on the cold marble slab but interpose a bad conductor, such as a fairly thick notebook.

There is another circumstance which often leads to faulty connections between the absorption tubes and between these and the neck of the combustion tube, namely, unequal size of the parts to be connected. In my apparatus the outer diameter of these is 3 to 3·5 mm. The difference in diameters of two parts which are to be connected should never exceed 0·5 mm., as otherwise there is a dead space between the glass and the rubber tubing which leads to more opportunity for errors to be discussed later.

In order to ensure approximate uniformity of the interconnected portions, I now make the neck of the combustion tube by sealing on a tube of Jena glass of the same quality of 3 to 3·5 mm. diameter to a combustion tube of 10 mm. external diameter and 400 mm. long, instead of by drawing out the latter.

I must now describe the absorption tubes with two revolving ground stoppers constructed by Ferdinand Blumer (Fig. 10). They consist of a thin-walled glass tube, about 135 mm. long, to hold the absorption agent, both ends of which can be connected at will by revolving glass cocks with the two side tubes, which are not constricted and are attached at right angles to the main axis and point in opposite directions. The tube filling can thus be completely protected from the outer air, and to that extent a very constant weight can be ensured. The revolving glass cocks are, however, difficult to clean and wipe ; this can be shown by turning them once, re-wiping and re-weighing, when it will be found that there is usually an alteration of weight amounting to 0·02 or even 0·05 mgm. This is caused by the necessary tap-grease, a small amount of which is easily pressed out and wiped off, or by dust particles which adhere to the grease. After consideration I concluded that by introducing ground joints instead of rubber tubing, either for interconnecting the absorption train or for connecting it with the neck of the combustion tube, the surface to be wiped would be considerably increased, and for this reason I decided, after many experiments with the very attractive Blumer absorption tubes, to revert to my own tubes, if only on account of the ease in handling whether by the beginner or by the expert. These remarks apply to our latitudes and climates. It is possible that in the tropics the Blumer tubes would offer advantages in spite of the drawbacks already indicated.

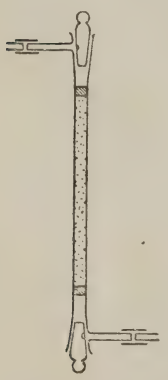

Fig. 10.—Blumer's absorption tube.

The Mariotte Flask

This serves to maintain a definite and easily adjustable reduced pressure in the absorption train to overcome the friction in the same to such an extent that approximately atmospheric pressure is maintained at the rubber connection between the combustion tube and the calcium chloride tube. As indicated in Fig. 3, *MFl*, it consists of a levigating bottle of $\frac{1}{2}$ to 1 litre capacity, into the lower tubulature of which a narrow glass tube of about 3 mm. diameter is fixed by means of a perforated cork stopper in such a manner that it can be rotated to form an adjustable one-armed syphon. If the bore exceeds 2 mm. it is sometimes difficult to fill the syphon with water on lowering it.

The glass tube is bent for this purpose at right angles at one end, and the short limb inserted into the cork. The long portion, which should reach at least to the upper neck of the bottle, is bent to one side at the end. Rubber stoppers are unsuitable for holding this tube, as they hold it too stiffly, but when inserted into a cork stopper any position is readily maintained. The upper tubulus of the levigating flask is provided with a doubly bored rubber stopper, one hole of which is closed with a suitable glass rod, which should be removed when the apparatus is not in use, in order to prevent water being driven out in case the temperature rises in spite of the vertical position of the syphon. Through the second perforation in the stopper, a capillary of at least 2 mm. bore, and which is bent twice at right angles, is inserted, as shown in the figure, in such a position that it reaches nearly to the bottom of the bottle. The free end of the capillary, which is directed downwards, is connected by means of rubber tubing with a small calcium chloride tube (Fig. 11), which is connected during analysis with the end of the soda-lime tube by means of ordinary rubber tubing.

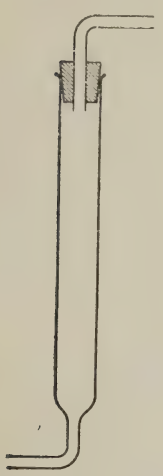

Fig. 11.—Calcium chloride tube with two connecting tubes bent at right angles. (Actual size.)

The Connecting Tubing

The air-tight connection of the calcium chloride tube, with the neck of the combustion tube on the one hand and with the soda-lime tube on the other, requires special consideration, because it was gradually found by many tedious experiments and failures that india-rubber, although an absolutely indispensable material for organic microanalysis, possesses, in the form in which it is available, many properties which render it unsuitable for the purpose, for firstly, it is hygroscopic: secondly, very often porous, or becomes porous during use through production of capillary cracks or injuries, and thirdly, it is permeable to carbon dioxide.

Its *hygroscopic properties* were very evident from the blank tests. If these were carried out without any precautions, the increase of weight of the calcium chloride tube was found to be 0·1 mgm., even when the potash apparatus showed no increase. This increase in weight of the calcium chloride tube did not occur when the rubber connection was previously carefully dried *in*

vacuo, or, still better, if it had been in position for some time on the hot neck of the tube whilst this was pre-heated. This led to the conclusion that the results were due to the hygroscopic character of the rubber.

Further experiments showed that it was not advisable to dry the tubing in this energetic manner before carrying out an analysis, as in that case hydrogen values were obtained which were rather low, particularly with substances with high hydrogen content. This fact is easily explained by absorption of water, during prolonged contact, by the previously dried rubber, which is not completely removed during the short period of the analysis by the current of hot air.

Further attempts to find a more suitable material for connecting the absorption tubes with one another and with the neck of the combustion tube finally led to a preliminary treatment of the rubber connections which reduced the previously mentioned drawbacks of the rubber colloid to such an extent that it is often possible, even without a Mariotte flask—that is, without any regulation of the pressure—to obtain satisfactory results, as will be shown later.

For preparing the rubber connections, one uses thick-walled rubber tubing of about 8 mm. outer diameter, and 2 mm. bore. A narrower bore is unsuitable, as the considerable expansion of the tubing, when passed over the side tubes, affects its soundness just as much as excessive and unnecessary constriction or pressure. The inner surface of the tubing must be perfectly smooth and without any trace of a seam. The colour appears to be unimportant. For connecting the neck of the combustion tube with the calcium chloride tube, lengths of 15 mm. are used, and for connecting the latter with the soda-lime apparatus, lengths of 20 mm.; these are placed in a small flask with crude molten vaseline, which is then evacuated with the water pump on the boiling water bath. As soon as the contents have ceased to foam, air is re-admitted in order to force the molten vaseline into the finest interstices in the rubber. This evacuation and re-admission of air is repeated until at the highest attainable vacuum no further bubbles arise from the tubing. The pieces of tubing are then allowed to drain whilst still warm, wiped without and within, and then provided with a stiff paper coating, in order to give them the necessary rigidity, and also to protect them from unnecessary and harmful constriction and pressure. This is done by cutting strips of writing paper, which are rather broader than the length of rubber tubing.

The whole surface of these strips is then covered with an adhesive, the best being starch paste, and after being thoroughly moistened the strip is then rolled round the rubber tube, beginning at one end, in such a manner that the paper covers the tube, without wrinkles and without any interspaces, five to ten-fold. After drying for twenty-four hours in a cool place the projecting ends of the paper cover are cut off with sharp scissors, and the prepared tubes are kept ready for use in the balance case. They must be wiped inside each time before use with a small wad of cotton-wool impregnated with a minute amount of glycerine, which is wound round the end of a wire. It is subsequently necessary to wipe through the tubing with a dry wad of cotton-wool free from loose fibres in order to remove any excess of glycerine.

It might be supposed that the above-mentioned unsatisfactory qualities of rubber connections could be avoided by replacing these by ground joints between the apparatus. This idea attracted me considerably at first, but I had to reject it during the first development of microanalysis, after considerable reflection, having realised that microanalysis in general is better served by avoiding ground joints. I was therefore exceptionally pleased to learn, in a communication from the late Professor Schrader, of Heidelberg, of March 17th, 1917, that he at first used ground joints, but convinced himself that success could only be attained with rubber connections which had been treated and handled according to my instructions. In spite of this, Ernst Müller and Hertha Willenberg, and others, have latterly used apparatus with ground joints.[1] I think that these innovations will ultimately be discarded, as rubber connections, whilst equally effective, are much cheaper than ground joints, which would probably necessitate the replacement of the whole apparatus in case of fracture. I have already referred to the greater difficulty in exact cleaning.

I wish to add that at present the firm of Paul Haack, in Vienna, provides tubing for this purpose, the quality and dimensions of which are excellent, and by using which the necessity for coating with paper after impregnation becomes unnecessary, as it is already sufficiently rigid.

On the Pressure and Speed Relations which are necessary in carrying out the Analysis

It follows from the remarks that have already been made that, in order quantitatively to convert an organic substance into the

[1] *Journ. f. prakt. Chem.*, 99 (1919), 34–44.

final combustion products, a certain minimal duration of contact of the gases and vapours which are produced with the red-hot portions of the tube filling is necessary, that is, that the speed of the gas current in the combustion tube must not exceed a certain maximum value. It was found by numerous analyses of substances which are recognised as combustible with difficulty, more particularly of cholesterin, anthraquinone and similar substances, that with a gas speed of 3 to 4 c.c. per minute the combustion is absolutely complete. A large number of blank experiments have shown that, in spite of the use of irreproachable oxygen and of matured rubber tubing for introducing the gases, both absorption tubes increase in weight if the pressure in these is seriously reduced. On the other hand, it was found that if the pressure in the absorption train, and more particularly in the connection between the neck of the combustion tube and the calcium chloride tube, was higher than the atmospheric pressure the figures which were obtained in the combustion of easily combustible substances were lower than those required by theory. This suggested that carbon dioxide and water were aspirated through the warm rubber connection in the former case and expelled in the latter.

Various experiments were carried out in connection with this matter, the results of which are sufficiently interesting to be communicated. Carbon dioxide contained in a gas burette over mercury was connected with rubber tubing, the end of which was closed by a glass rod. After a few minutes a reduction of the volume could be observed. If the experiment lasts sufficiently long, the gas vanishes from the enclosed space until only a small amount is left in spite of the production of a reduction of pressure, which often amounts to more than 200 mm. of mercury. This phenomenon takes place to a slightly less extent and in a rather longer time, when tubing is used which has been treated with crude molten vaseline *in vacuo* in the manner already described. If, on the other hand, air containing carbon dioxide is enclosed over mercury in a gas burette, with a piece of tubing which has been exposed for a considerable time to carbon dioxide under pressure, and through which air has been drawn for a few seconds only in order to remove carbon dioxide, at any rate from its bore, one observes an alteration of the mercury level of 1 to 2 mm. only, even after several hours. It follows that the carbon dioxide which is absorbed by rubber is liberated extremely quickly by passing through it a current of pure air, that is,

the migration of the carbon dioxide in the rubber colloid in the direction from a higher to a lower partial pressure is very rapid.

These experiments show that the selective absorption of carbon dioxide by rubber, and its capacity for liberating it again in the direction of a lower concentration is merely reduced by impregnation with vaseline, whereas its ordinary capacity to absorb and liberate water vapour is almost entirely removed by this process ; for tubing treated in this manner, when tested by a blank experiment, transmits at most 0·02 to 0·04 mgm. of water to the calcium chloride tube in spite of being heated by the hot neck of the combustion tube.

From these experiments it follows also that any carbon dioxide which penetrates into the body of the rubber connecting tubes during the short period of the combustion is almost quantitatively removed by the following stream of air free from carbon dioxide. From this standpoint pressure equalisation by the Mariotte flask appears to be entirely unnecessary. It is otherwise, however, with the imperfection of the connection which occurs after frequent use, even with impregnated rubber tubing, and often occurs suddenly and unnoticeably. In such cases it is clear that if there is a positive pressure inside the tubing loss of carbon dioxide must occur, and this is in accordance with the results of blank experiments and of analyses. On the other hand, in such cases, if the pressure within is below that of the atmosphere, moist air will be absorbed.

In this case we are dealing, therefore, with losses or increases caused by leakage of the rubber connections, whether through capillary cracks, which have been produced in course of time, or through definite porosity of the tubing. In either case the tubing connecting the neck of the calcium chloride tube is more exposed to injury than that between the two absorption tubes on account of the greater degree of heating.

By the use of the Mariotte flask we are enabled accurately to absorb the products of combustion of the substances to be analysed without loss and without erroneous increase in the weight of the absorption tubes, even if the rubber connections between the absorption tubes and the combustion tube are faulty.

The Mariotte flask is thus a safety device which enables us to carry out analyses untroubled by doubts regarding the absolute freedom from leakage in the connections between the absorption

tubes and the combustion tube,[1] and also ensures certain other advantages :

1. After completing the combustion, the amount of air which is aspirated can be measured by means of the water which runs away, thus enabling one to be sure that all the water produced by the combustion is driven into the calcium chloride tube. This could not be effected in the case of substances rich in hydrogen without the Mariotte flask, as it would hardly be possible to recover quantitatively any water vapour which might be driven into faulty tubing by the excess pressure.

2. It affords a convenient means of calibrating the bubble-counter.

3. It is used for aspirating a slow current of air through the absorption tubes after these are filled, and

4. It enables us thoroughly to ignite the empty part of the tube starting from the open end.

Considering the pressure relations in the whole system, it is clear that the gas pressure, corresponding to the head in the pressure regulator, will exist from that point up to approximately that point in the combustion tube where the three-fold asbestos choking plug behind the lead peroxide is located, and will be approximately constant up to that point. In consequence of the considerable gaseous friction caused by the choking plug, this only allows equal quantities of gas to pass in equal times, and also gives rise to a sudden diminution of pressure behind it, which continues unchanged up to the first constriction of the calcium chloride tube, and may be considered to be parallel to the axis of abscissæ if we consider the barometric pressure to be represented by the ordinate zero in a right-angled system of co-ordinates. Then, if the neck of the combustion tube were open, that is, if no absorption train were connected with it, the pressure diagram between the choking wad and the end of the neck would correspond approximately with the axis of the abscissæ. If, however, we connect up with the absorption train, then, in consequence of the inevitably high gaseous friction in the tubes, the velocity of the gas current will be less, and in consequence of the increased inner pressure the pressure curve will be parallel to the axis of the abscissæ with a positive ordinate value. In order to reduce this positive ordinate value to zero at this point, that is, in order to

[1] It is well worth noting that A. Schoeller (*Zts. f. angew. Chem.* (1921), 34th year, No. 93 of November 22nd, p. 582) emphasises the advantages of the Mariotte flask even when using Blumer's absorption tubes, which offer only very slight resistance to the gas current.

displace the pressure curve between the choking wad and the first capillary constriction of the calcium chloride tube so that it coincides with the axis of the abscissæ, it is necessary to exert a constant reduced pressure at the end of the soda-lime apparatus. It was such considerations which led to the use of the Mariotte flask, the adjustment of which, in order to establish the necessary pressure and velocity relations, may be embodied in the following three rules :

I. By connecting the neck of the combustion tube with the Mariotte flask the particular bubble frequency is determined at which 3 to 4 c.c. of gas pass through the tube section in one minute, by measuring in a graduated cylinder the water which flows away. This gas velocity is obtained by varying the adjustment of the pressure regulator and altering the adjustment of the syphon of the Mariotte flask accordingly.

II. After disconnecting the Mariotte flask, the pressure regulator is so adjusted that the bubble frequency determined according to (I.) is again obtained. This corresponds to one particular adjustment of the pressure regulator, which must be maintained for the particular combustion tube thereafter.

III. If the absorption train is now connected without the Mariotte flask at the commencement of the combustion, the bubble frequency is diminished. By connecting the Mariotte flask with the absorption train and adjusting the syphon, the gas friction in the absorption train is neutralised to such a point that the bubble frequency again assumes the value obtained in (I.) and (II.) without absorption train. It should be noted, further, that an excess of suction of 5 to 10 mm. by means of the Mariotte flask is less objectionable than a positive pressure in the absorption train, which easily leads to losses of carbon dioxide and water, whereas, if the rubber connections are in a satisfactory condition, a somewhat increased suction still leads to correct results, as the impregnated tubing evolves practically nothing, and as the inward pressure of the elastic rubber connections to some extent compensates for the reduced inner pressure in the absorption train and prevents small leakages. If, on the other hand, the rubber connections are somewhat damaged, more particularly that between the neck of the combustion tube and the calcium chloride tube, then excessive suction by means of the Mariotte flask will undoubtedly lead to values of carbon and hydrogen which are too high.

It follows clearly from the above that the condition of the

rubber connections is of great importance, and that the defects of such connections must be reduced by impregnation on the one hand and by suitable use of the Mariotte flask on the other. As, in consequence of the Great War, the tubing at our disposal steadily becomes worse in quality, it seems to me particularly important to emphasise these points at the present time.

Looking to the future, in which the best quality of rubber tubing will again be available, I consider that it would be better to dispense with the Mariotte flask rather than with the impregnation of the tubing. All that is necessary in this case, in order to ensure that no moist air has access to the soda-lime tube, is to attach to it, by means of rubber tubing, the small vertically suspended calcium chloride tube with two side tubes bent at right angles, which is otherwise used for the same purpose at the end of the rubber tubing connecting it with the Mariotte flask. It must be emphasised that the side tubes of this calcium chloride tube (Fig. 11) must not be provided with capillary constrictions, or any other resistance causing gaseous friction, as any pressure increase is clearly to be avoided. For the same reason, it is not advisable, and quite superfluous, to add a bubble-counter at this point.

The result of all the above-mentioned work is an apparatus in which, as a result of theoretical considerations, a number of conditions have been fulfilled in a manner much more complete than has so far been the case in ordinary macroanalysis. It causes the gases to issue from the combustion tube at a perfectly definite velocity which is easily established, and which is determined empirically, and thus ensures that each gas particle remains in contact with the admittedly suitable tube filling (universal filling) for a definite and constant period of time. This is attained by the choking plug at a point near the exit of the gases from the combustion tube. Its effect differs essentially from that of a pinch-cock which regulates the entrance of the gases to a combustion tube in which there is no gaseous friction, for this latter arrangement only ensures regularity of the gas stream, but not a uniform time of contact with the red-hot tube contacts. As the above-mentioned effects of the choking plug are only possible when the pressure remains constant, and as this result is difficult to maintain with certainty by means of a pinch-cock, it was achieved by the use of a small glass gas-holder, the pressure regulator, which enables one to control the desired gas velocity by establishing the necessary pressure and renders an excess pressure quite impossible.

Blank Tests

These are necessary in order to ensure that the apparatus is working satisfactorily, and also in order to detect any defects.

A blank test is carried out exactly in the same manner as an analysis, the only difference being that no boat is used, and that the Mariotte flask is also dispensed with, because only a minute proportion of any increase in weight of the soda-lime tube can be lost in consequence of an absence of equalisation of the pressure by that apparatus. As compressed oxygen made from liquid air and clean air from without the laboratory are always used to fill the gas-holders, and as matured tubing only is used for the connections, the blank test now only serves the purpose of discovering whether a freshly filled tube, or one which has not been used for some time, has been sufficiently pre-heated. In the case of freshly filled tubes, in particular, it is often observed that, even after heating for several hours, considerable increases in the weight of the absorption tubes occur. In such cases the increase in weight of the soda-lime tube is doubtless to be ascribed to the lead peroxide, which not only absorbs water, but also obstinately retains for a long time the last fractions of nitric acid which has been used for its purification. For this reason we heat newly filled tubes for at least a day in a rapid stream of air or oxygen before the first blank test is made. Since using lead peroxide asbestos a newly filled tube can be thoroughly cleaned in one to two hours. Lead chromate also seems to give rise to such increases of weight if it has not been thoroughly ignited in a dish before it is used for filling.

Increases in weight of the absorption tubes are not necessarily a proof of faulty condition of the tube filling of the gases or of the delivery tubes, but may also be caused by other circumstances which merit particular attention. The most important of these are excessive treatment with glycerine of the connecting tubes, and insufficient or faulty wiping of the absorption tubes after finishing the test. This latter circumstance is often the cause of analytical results which are too high obtained by beginners. Apart from these two serious errors we must also mention two effects which may even baffle the expert. One of these is a temperature in the balance room which is essentially lower than that in the combustion room ; this leads to the aspiration of moist air into the apparatus on cooling. The second is, insufficient corre-

spondence of the drying power of the filling of both absorption tubes with the filling of the U-tube attached to the bubble-counter. This effect was unrecognised by us for a long time, as we then changed the filling of our apparatus at relatively short intervals. The conditions are, however, quite altered, if filled apparatus is left unused for some time, even though it is protected by rubber caps. The water content of the whole filling then becomes equalised so that, for example, in a calcium chloride tube which has already been used, water wanders from the point of entry of the gas stream throughout the tube towards the exit, and on again using the tube, the gas stream which has passed through it no longer acquires that degree of dryness which would originally have been obtained, so that an attached, freshly filled soda-lime tube shows a more or less serious increase of weight during the blank test. If, in such cases, one repeats the blank test under otherwise correct conditions, but interchanging the absorption tubes, one will find that the soda-lime tube which, in this case, is attached to the neck of the combustion tube, will show an increase of weight, but that the calcium chloride tube will not, and an analysis carried out with such absorption tubes, but otherwise correctly, will show a hydrogen value which is seriously low, and a carbon value which is much too high. If, on the other hand, the filling of the calcium chloride tube is renewed, whilst in the soda-lime tube, which has been repeatedly used, the soda-lime only is replaced and not the calcium chloride, and if the U-tube which has been used repeatedly has not had its filling renewed, then with such apparatus a blank test will show a considerable increase in weight of the newly filled calcium chloride tube, whilst the soda-lime tube will remain constant. In this case, however, approximately the same results will be obtained in a blank test with transposed absorption tubes, and an analysis carried out with such tubes, though otherwise correctly conducted, will show hydrogen values which are too high and carbon values which are too low.

For this reason we always use calcium chloride of groat size for filling both the absorption tubes and the U-tube with the bubble-counter ; this calcium chloride is always kept in the same bottle with a ground stopper smeared with vaseline. One should never spare the trouble, after leaving the apparatus idle for some weeks, of refilling both the absorption tubes and the U-tube with the bubble-counter. This small precaution avoids many unpleasant failures.

Various Considerations

I will now discuss various matters which are of little interest to the beginner, who wishes to acquire the methods, but which were decisive in the development of the microanalytical determination of carbon and hydrogen. They are concerned firstly with the influence of defective tube connections on the analytical results under various conditions, and secondly with the relations between pressure, velocity of the gas current, and the construction of the absorption tubes.

The errors which result in the weight of the soda-lime tube through the use of rubber connecting tubing which is not impregnated, and which is rather porous but in no way seriously damaged, may amount, when the Mariotte flask is not used, to 0·05 mgm. It is clear that under such circumstances useful analytical results can only be obtained with quantities of material under 5 mgm. if the errors happen to compensate one another, that is, if the gases are not perfectly pure or if the delivery tubes for these have not been matured and are good new rubber tubing, or finally if absorption tubes are used which increase in weight during cooling by an amount which is approximately equal to the error produced by omitting the Mariotte flask.

On using larger amounts of material of about 10 mgm., the influence which has just been discussed will be so small that the deviations of the values found from the theoretical values will be well within the permissible margin of error.

The following considerations emphasise the correctness of this view. If 2·727 mgm. of material are used, then 0·01 mgm. of carbon dioxide correspond to 0·1 per cent. of carbon. As with practice the weight of the absorption tubes can be reproduced with a constancy of 0·005 mgm., it is clear that considering all the effects which I have referred to, correct analytical values will be obtained by the combustion of roughly 1·000 mgm.—for if 1·363 mgm. are used, an error of 0·005 mgm. in the weight of the soda-lime tube will only affect the carbon value by 0·1 per cent.

The same method of calculation shows how much less the various sources of error affect the analytical results when the quantity used is increased. When 11·908 mgm. of material are used, an error in weight of 0·04 mgm. of carbon dioxide is required in order to produce an error of 0·1 per cent. in the carbon, and if 13·655 mgm. are used, an error of 0·1 mgm. carbon dioxide causes an error of 0·2 per cent. only in the carbon value. Under

these circumstances, we may pass hot, freshly prepared oxygen into the gas-holder through new rubber tubing, which has not been matured, without any fear that the permissible error will be appreciably exceeded, if at all.

Although these considerations favour an increase of the amount of substance used for the analysis, there are others which render it preferable to use small amounts of the order of 2 to 5 mgm. This is particularly the case if the substances to be analysed contain halogen and sulphur as well as nitrogen, as when such small quantities are used the quantitative absorption of the gases produced from these elements, which would otherwise be absorbed by the soda-lime tube, takes place with such certainty, if all the necessary conditions are present and if my filling is used, that the latter remains effective for a much larger period than the life of the combustion tube (200 to 300 analyses). If, on the other hand, larger quantities were used for the combustion, the tube filling would be exhausted at an earlier stage. Apart from the life of the tube and of its filling, the use of small quantities of material is justified by the fact that the combustion of larger quantities entails a longer period of attention by the analyst, and his consequent impatience often leads to serious errors.

It is quite clear that if the Mariotte flask is not used the pressure on the rubber connections increases with increase of the velocity of the gases, and that the difference between this pressure and the barometric pressure decreases with decreasing speed of the gases traversing the system. Consequently with an extremely small gas speed of about 1 to 2 c.c. per minute one may dispense with the use of the Mariotte flask, even though the rubber connections are not quite perfect, for at such a speed the frequency of the bubbles in the bubble-counter remains almost constant, whether the absorption train is attached to the combustion tube or not. The use of so slow a gas current, of 1 to 2 c.c. per minute, cannot, however, be recommended, because the transference of the products of combustion along the tube in the direction of flow is difficult to achieve, and they therefore tend to travel back in the opposite direction, as is particularly obvious in the case of substances which sublime. If the gas velocity amounts to 3 to 4 c.c. per minute, which is the most favourable speed, the pressure increase at the rubber connections is appreciable, as is clearly shown by the very marked reduction of the bubble frequency after connecting the absorption train. The slightest faults in the rubber connections, such as small capillary apertures, will then allow

carbon dioxide to escape in the manner already indicated. Consequently, although in such cases the blank test may yield absolutely correct values, that is, no increase of weight of the absorption tubes, and particularly of the soda-lime tube, carbon values will be obtained when the Mariotte flask is not used which are several tenths of a per cent. lower than the theoretical result.

The matter is quite otherwise if, in spite of some care, the blank test still shows a small increase in weight of the soda-lime tube, even after the combustion tube has been pre-heated for some time, in consequence of a source of carbon somewhere in the system. This may be due either to the admission of air containing the vapours of organic solvents in place of fresh air, or the use of delivery tubing which has not been carefully matured, or to the fact that the lead peroxide or lead chromate have not been completely freed from their acid impurities. In such cases the blank test, whether the Mariotte flask is used or not, will show an increase of weight up to about 0·03 to 0·05 mgm. ; analysis of a definite substance carried out under conditions which are otherwise satisfactory will then show a carbon value which is a few tenths of a per cent. too high, in spite of the use of the Mariotte flask, whereas, when the Mariotte flask is not used, the same substance will show a carbon value which is approximately theoretically correct. It is clear that such correct values are only due to the circumstance that the two errors compensate one another.

There is a second method, other than the reduction of the gas velocity, of reducing the internal pressure in the rubber connections, namely, a reduction of the gaseous friction in the absorption train. This may easily be effected by slightly increasing the diameter of the capillary constrictions in the side tubes and the capillary openings in the glass walls of the absorption tubes which separate the filled inner portion from the ante-chambers. Such widening of the gas passages at these points necessarily impairs the constancy of weight of the tubes. It is true that one may reduce the friction in the tubes by means of comparatively large openings as described without seriously impairing the constancy of weight if the ante-chambers are enlarged. This method of compensation can, however, only be applied to a limited extent for various reasons, as an increase in the size of the ante-chambers causes a number of other disadvantages. For instance, the expulsion of the water from the ante-chamber of the calcium chloride tube into the central portion then requires more attention

during analysis. A large ante-chamber at the other end, which would entrap more or less moist air whilst cooling in the balance case before the analysis, would give rise to an appreciable increase in weight of the soda-lime tube through diffusion of this moisture into it during analysis. The only ante-chamber which can be considerably enlarged without any prejudicial effect is that at the exit end of the soda-lime tube. Tubes constructed in this way show quite exceptional constancy of weight. To the ante-chamber at the inlet end of the soda-lime tube, that is, to the chamber within the stopper, the same considerations apply as to the chamber in the stopper of the calcium chloride tube. It has been found by experience that if the diameters of the constricted points in the absorption tubes measure from 0·2 to 0·3 mm., the capacity of the ante-chambers should be not less than one-twelfth of that of the inner chamber which holds the filling if a sufficient constancy of weight is to be attained. With such dimensions of the capillary obstructions to the gas current, the increase in pressure inside the apparatus is no longer very considerable ; for the bubble frequency then suffers only a very slight reduction when the absorption train is attached to the neck of the combustion tube, and the analytical results which may then be obtained without using the Mariotte flask are tolerably good if the other conditions are satisfactory. The carbon value is then usually still quite passable, although slightly low, but the hydrogen value is usually less satisfactory, because it is too low, for any water which condenses at the connection between the neck of the combustion tube and the calcium chloride tube is either driven into the hair capillaries of the rubber tubing, or diffuses as vapour in consequence of its low molecular weight as compared with that of carbon dioxide (18:44).

These considerations show that in the construction of the absorption tubes a number of conditions, some of which are opposed, must be satisfied in order to attain complete success. It is remarkable that I constructed the first absorption tubes in 1911 more or less instinctively to a pattern which was completely in accordance with their purpose, and that it was only the various subsequent modifications which led to the knowledge which has just been communicated.

If the capillary openings in the absorption tubes are of smaller diameter than 0·2 to 0·3 mm., that is to say, 0·1 mm. or less, the other dimensions of the tubes being maintained, then the conditions become less favourable for the quantitative absorption of the combustion products, in consequence of the increased internal

pressure which shows itself by a reduction of the bubble frequency. This is particularly true of the two capillary constrictions of the calcium chloride tube at the end adjacent to the neck of the combustion tube, because if the diameter of these is less than 0·2 mm. they are very often obstructed by drops of condensed water ; the interference with the regular motion of the gas stream thus caused usually leads to consequences which make the whole success of the analysis doubtful. If, however, we completely remove any excess pressure within the absorption train and the rubber connections, all these difficulties are removed completely. This is effected by the Mariotte flask which suitably reduces the internal pressure even though the absorption tubes have capillary openings which are rather wider and are connected by somewhat doubtful rubber tubing which may be slightly cracked. Such defects in the tubing are often unnoticeable, and I therefore consider the use of the Mariotte flask to be the most reliable way of obtaining correct results providing always that the other conditions are satisfactory, that is, more particularly that the blank test gives absolutely faultless results whatever the relative positions of the absorption tubes.

Summarising the above considerations we find that the micro-analytical determination of carbon and hydrogen can be carried out easily and accurately if, apart from the use of pure gases and suitable tube filling, the following conditions are strictly observed :

1. The degree of dryness of the gas entering the combustion tube must be equal to that of the gas which leaves the absorption tubes, *i.e.*, the calcium chloride in the U-tube and in the absorption tubes must be in the same condition.

2. Each particle of combustible vapour must remain in contact with the hot tube filling for a time sufficient for the completion of the desired reactions. This is secured on the one hand by the choking plug, which permits equal gas volumes to pass in equal times, and on the other hand by the pressure at which the gases enter the combustion tube when the pressure regulator is suitably adjusted. The gases should leave the absorption train at a rate of 3 to 4 c.c. per minute.

3. It is necessary to avoid both loss of the products of combustion and entry of products from without into the absorption tubes. The fulfilment of these conditions depends—

(*a*) On careful selection and correct treatment of the rubber connections,

(*b*) on equal dimensions of the ends of the absorption tubes, and the neck of the combustion tube, and

(*c*) on approximate equalisation of the pressure inside the rubber connecting tubes with the atmospheric pressure. This is ensured by suitable adjustment of the Mariotte flask.

The Preparation of Materials for Analysis

A. Solid Substances

As the majority of substances which are analysed are not hygroscopic, the weighing of the material can, in these cases, be conducted without further complication in an open platinum boat. This latter can be obtained of the specified dimensions from the firm of Heraeus, of Hanau-a.-M. It is advisable in the case of materials which damage platinum—for instance, those containing much halogen, lead, or more particularly arsenic—to use small boats of porcelain or resistance glass. The boat should be boiled with dilute nitric acid in a test tube before each analysis, and then ignited in a flame whilst suspended from a platinum hook. It is then placed on a circular block of copper of 40 mm. diameter, with a slightly concave upper surface on which the boat attains the temperature of the balance room in a few seconds. These copper blocks are best placed in small desiccators in which the clean, weighed boat, with or without the substance, is kept under cover for further use.

The prepared platinum boat is removed from the desiccator with the copper block into the neighbourhood of the balance pan. Both are placed near the left balance pan, and the boat is then transferred to the pan by holding it by the handle with clean platinum-pointed forceps. The copper block must be removed before weighing, as otherwise any slight temperature difference between it and the interior of the balance may give rise to zero point deflections. After weighing, which must be carried out with the greatest care to within 0·001 mgm., the boat is transferred to a dusted page of the notebook and filled with a suitable quantity of the material to be analysed with the help of a knife point. Usually not less than 3 mgm. are weighed, and more than 5 mgm. should not be used. It is not only unnecessary, but sometimes even disadvantageous to use larger quantities for the combustion, as the only result is to necessitate the attention of the analyst for a longer time. In special cases one can manage

with even smaller quantities than 3 mgm. ; if a blank test shows that all the arrangements are in faultless order it is even possible to obtain correct results with less than 2 mgm. of material. The smallest amount with which quite good microanalytical results have so far been obtained was about 1 mgm.

Before replacing the filled boat on the balance pan it is once

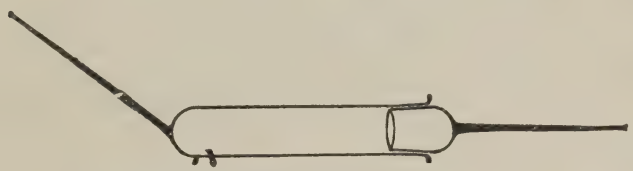

Fig. 12.—Weighing bottle. (Actual size.)

more held with the forceps whilst its sides and bottom are brushed with a sable brush which has previously been carefully freed from dust by shaking. The filled boat must be weighed with the same care and exactitude as the empty boat. The copper block is then again placed near the left weighing pan, the boat placed on the block, and both then placed in the desiccator.

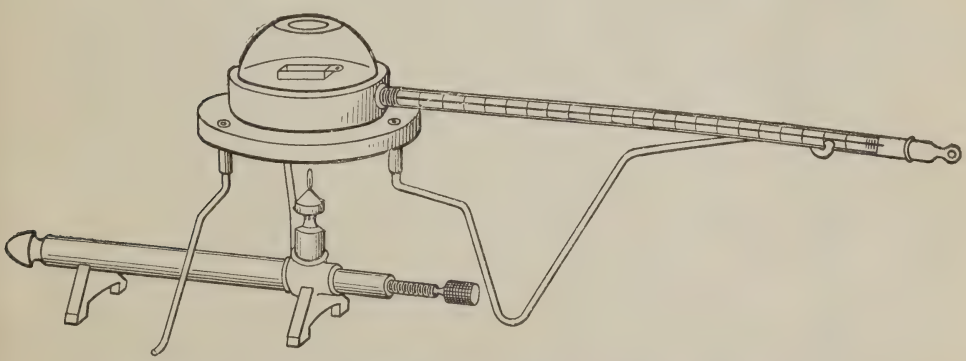

Fig. 13.—Drying block with micro-burner and thermometer, in use. ($\frac{1}{2}$ actual size.)

In the case of hygroscopic substances it is necessary to weigh both the empty boat and the filled boat in a weighing bottle (Fig. 12). On account of the high specific heat of glass the handles of this weighing bottle are very thin, in order to reduce the effect of holding it to a minimum. The weighing bottle itself should not be dried either in the desiccator or at a high temperature. It is best kept in the balance case, where it always assumes the correct temperature and degree of dryness for weighing.

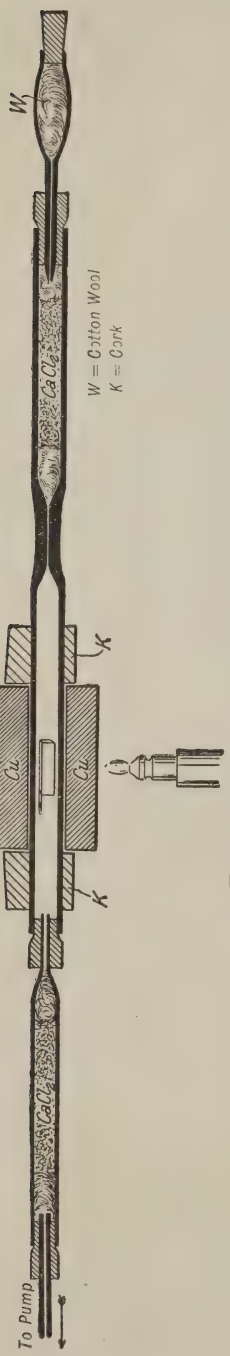

FIG. 14.—Micro-desiccator. ($\frac{1}{2}$ actual size.)

When it is necessary to dry hygroscopic substances *in vacuo* over sulphuric acid it is best to place the undried substance in the previously weighed boat, and then introduce it into the vacuum, for such small amounts dry more quickly under these conditions, and renewed absorption of water vapour is avoided by not having to fill the boat subsequently if care is taken that the boat is rapidly introduced into the weighing bottle, after which this is immediately closed.

In cases where it is necessary to dry at higher temperatures under ordinary pressure the so-called drying block (Fig. 13) is very useful. This is a copper or aluminium block of rather larger dimensions than the weighing blocks already described. Like these, it is provided with a concave upper surface, and also with a radial socket into which the bulb of a thermometer is inserted with the help of a strip of asbestos paper. The block rests on a ring of uralite, which is provided with three feet, which raise it to a height of 5 cm. above the bench top. One of these feet has a prolongation which serves as a support for the projecting end of the thermometer.

The block is heated by the non-luminous flame of a micro-burner placed on the bench under the block. On account of its peculiar shape, we call this burner a "Dachshund" burner. Its essential characteristic is that the gas issues from a steatite jet, such as is used in acetylene burners. This jet is provided with four lateral apertures which ensure the admixture of air and thus render the flame non-luminous. A small adjusting screw enables the flame to be reduced to the size of a pin-head without extinction. By this means it is possible to maintain a constant temperature to within a few degrees. If used, for instance,

for the determination of water of crystallisation, the substance is weighed into the boat with an accuracy of three decimal places, placed on the concave surface of the drying block and covered with a glass evaporating dish. Up to 150° C. the readings of the thermometer are sufficiently accurate. At higher temperatures the substance to be dried is no longer at the temperature which the thermometer indicates. This drying block is also very useful for preparative work—for instance, when it is necessary to dry

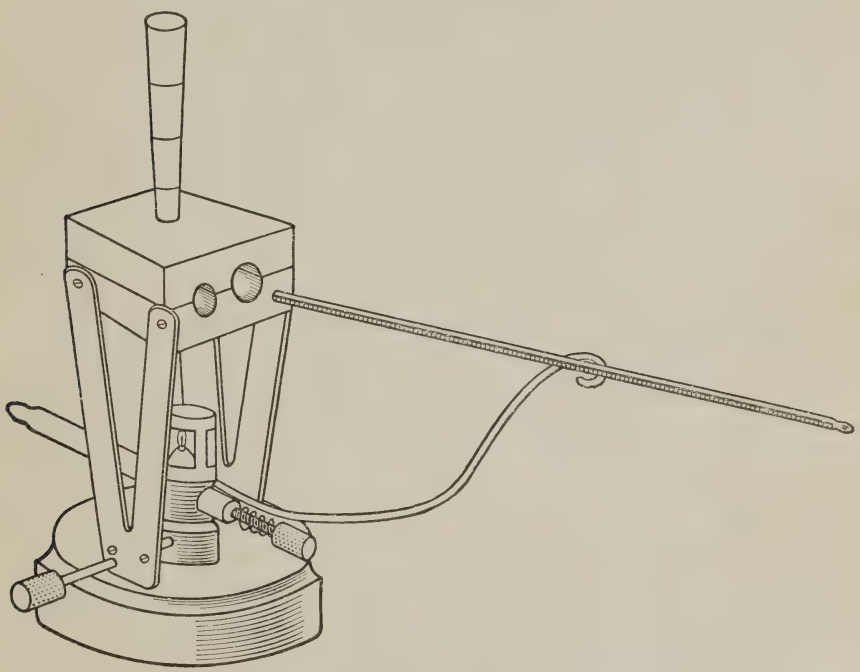

Fig. 15.—Regenerating block. ($\frac{1}{2}$ actual size.)

small amounts of crystallised material which are still moist before determining the melting point.

In special cases it is necessary to dry *in vacuo* at higher temperatures. This operation is most simply carried out in the so-called micro-desiccator (Fig. 14). This consists of a tube 240 mm. long and of 10 mm. external diameter, the walls of the central portion of which have been thickened in the flame to such an extent that the bore is reduced to a hair capillary only. Into one half of this tube several layers of firmly compressed cotton-wool are inserted and followed by granulated calcium chloride about 50 mm. long, which is held in place by a further layer of cotton-wool. The

opening of this half of the tube is closed with a suitable piece of rubber tubing into which a fine thermometer capillary is inserted. The other end of this is expanded to form an olive-shaped bulb, which is filled with compressed cotton-wool. The empty half of the main tube is intended for the reception of the boat with the substance to be dried. The open end of this half is also closed with rubber tubing through which is inserted the end of a small tube filled with calcium chloride, the other end of which is connected with a pump. If the system is now evacuated, the pressure drops in the chamber containing the boat with the substance to be dried to the lowest value attainable by the Sprengel pump, assuming that the capillaries are sufficiently fine. They are only intended to permit a minimal but constant movement of the gaseous contents of the drying chamber by the entry of extremely small amounts of dry air. The substance in the micro-desiccator is heated by insertion of the latter into a copper block (regenerating block) which is also used for drying the halogen tubes and is described in connection with these (Fig. 15).

In order to prevent the micro-desiccator from revolving about its axis, and thus upsetting the boat, the half containing the substance to be dried is held between two corks which fit exactly and which prevent any such circular motion by being pressed tightly against the copper block. Flat surfaces are also worked with a file on both corks which enable the micro-desiccator with boat and the contained substance to be laid on the bench top without rolling. After stopping the pump, one waits for a few minutes in order to allow pressure equilibrium to be re-established, transfers the warm micro-desiccator into the neighbourhood of the balance, removes the calcium chloride tube with its rubber tube from the opening, withdraws the platinum body with a platinum hook, and removes it with the help of platinum-pointed forceps to the weighing bottle which has already been opened and replaces the stopper immediately. After waiting for the necessary time of about five minutes, the final weighing is made.

B. Liquids

For use in the combustion of liquids, capillaries of about 1 mm. bore are prepared by drawing out old test tubes. These are further adapted with the help of a small, almost luminous Bunsen flame. The capillary is held in the hands at both ends, and then melted in the middle as in the accompanying drawing (Fig. 16), until a drop of molten glass (*a*) is formed. This is then drawn out

to a solid rod (*b*), about 25 mm. long, which is further heated till it separates in the middle (*c*). The whole capillary is thus divided into two similar halves, each of which consists of a capillary open at one end provided with a solid handle. The end of the handle is then melted to a small sphere. A small crystal of potassium chlorate is then introduced into the sealed end of the capillary, and is fixed there by careful melting (*d*). The capillary is then softened at a distance of about 6 mm. from the closed end, and quickly drawn out in the flame to a hair capillary about 10 to 15 mm. long, the end of which is opened by breaking (*e*). It is necessary to assure oneself before weighing, with the help of a lens, that there actually is a capillary bore. After weighing the empty tube with an accuracy of three decimals air is expelled by carefully warming the empty portion, which must not be sufficiently hot to melt the potassium chlorate, and the open end is inserted into the liquid, which is drawn in as the tube gradually cools. The liquid which has entered may be forced to the other end, on to the potassium chlorate, by holding the capillary point upwards between the thumb and forefinger, and striking the hand sharply on to the knee. The operation is most easily and rapidly effected by holding the filled capillary with the three first fingers in such a manner that its point is directed towards the hollow of the hand, and then using the extended arm as a centrifuge by

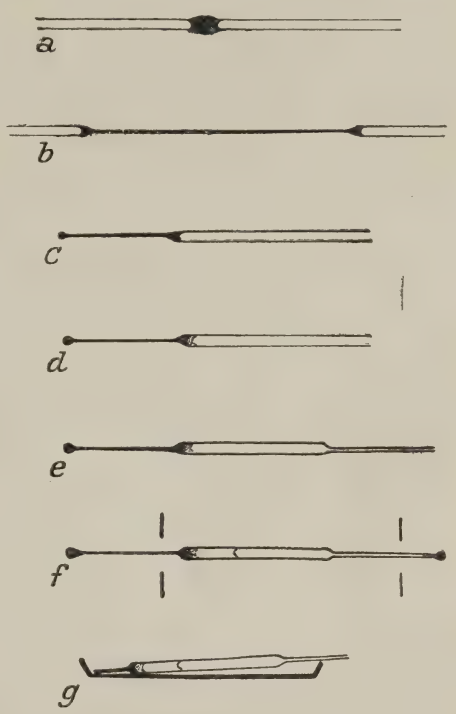

FIG. 16.—Preparation of the capillary for weighing liquids. (Actual size.) *a*. Production of a glass drop in the middle. *b*. Drawing out the same. *c*. One half produced by sealing off in the middle. *d*. After melting the introduced crystal of potassium chlorate on to the floor of the capillary. *e*. After drawing out the open end to a capillary (first weighing). *f*. After introduction of the liquid and sealing. *g*. The capillary after removing the handle and breaking off the point, lying on platinum foil at the moment of introduction into the combustion tube.

rotating it about the shoulder. This leads to the desired effect after a few revolutions. Traces of the liquid still remain in the capillary and on the walls of the empty portion. These must be removed by repeatedly and quickly drawing the empty portions of the capillary through the flame, as otherwise loss would occur on opening the same. The fine end of the capillary is sealed to a small sphere, and the whole is then weighed (*f*); the difference of the two weighings gives the weight of the included liquid with an accuracy of three decimal places.

Liquids can be weighed as above described, not only for the determination of carbon and hydrogen, but also for that of nitrogen, halogen and sulphur. For halogen determinations the potassium chlorate must be replaced by ammonium nitrate. For all determinations, with the exception of that of nitrogen, the substance is introduced into the combustion tube by placing in the opening of the latter a long narrow freshly-ignited strip of platinum foil on which the capillary is placed after first removing the handle, and finally, at the last moment, the extreme end, with the thumb nail, and then pushing it into the required position.

For the nitrogen determination the open capillary is placed in an oxidised coil of copper gauze, as will be described later.

Quite recently, in conjunction with Dr. Georgios Petridis, of Alexandria, I succeeded in effecting a further improvement which enabled us to obtain correct analytical results, even with very volatile and low-boiling liquids, by means of slight alterations. We found it necessary, in these cases, to give both the handle and the capillary a length of 50 mm. The capillary is filled in an almost horizontal position by dipping the point into the liquid and immersing it in a freezing mixture contained in a watch glass after warming the wider portion with the fingers. The capillary is then transferred to a centrifuge, the bucket of which is filled with the freezing mixture, is centrifuged for a short time, and the open point sealed whilst it is still in the freezing mixture. In this case the capillary is introduced into the combustion tube with the point closed and the handle still attached. It is left sealed in order to avoid loss at the moment of introduction, and the handle is retained in order that when the capillary is opened later, it shall not be forced back on to the stopper of the closed combustion tube by the rush of escaping vapour. After the combustion tube has been closed with a rubber stopper and the oxygen current has been properly established, that part of the tube containing the liquid is cooled in a freezing mixture, and the sealed point of the capillary

is opened by simply placing a Bunsen burner below it. The pressure within the capillary is sufficient to open the point gently as soon as it is softened. On removal of the freezing mixture, the contents of the capillary gradually escape. Finally, the portion containing the potassium chlorate is heated for a short time, in order to cause the combustion of the final traces of material. In this way we were able repeatedly to analyse ether correctly. We also regularly succeeded in analysing ethyl chloride, but the values found were lower than the theoretical values, which I am inclined to ascribe to impurity of the material.

The Course of the Analysis

To save time, it is best to commence by removing the rubber stopper at the mouth of the combustion tube, cleaning the mouth with a little cotton-wool wrapped round a match, closing it again, and then preheating the tube in a current of air with the burners of the tube furnace only slightly turned on. The hollow mortar is heated with the movable burner until the cymene boils. The front and side doors of the balance case are then opened, the absorption tubes, which have probably been filled on the previous day, are wiped with moist flannel and then with the two chamois leathers, and are placed on the stand near the balance case to cool. The substance is now weighed by weighing the boat both empty and when filled. In the case of hygroscopic substances which have been exposed on the previous day to a long drying operation, the boat with the substance is once more weighed in a weighing bottle, taking any alteration of the zero point into account, and any increase of weight is thus determined and deducted in subsequently calculating the hydrogen value.

Whilst the absorption tubes are cooling, the flames of the tube furnace are enlarged until the tube is at a dull red heat. The empty portion is heated with the movable burner commencing at a distance of 70 mm. from the rubber stopper, the air current is replaced by a current of oxygen and with the help of a watch the number of bubbles passing in ten seconds is noted. If necessary, the pressure regulator is readjusted in order to maintain the necessary gas velocity of 3 to 4 c.c. per minute, after which the bubble frequency per ten seconds must again be determined.

In weighing the absorption tubes, it is necessary to ensure that the soda-lime tube more particularly remains hanging in the glass balance case for about two minutes before being finally weighed. The weighed absorption tubes are interconnected at the balance

with the properly impregnated piece of rubber tubing, and the rubber connection of the calcium chloride tube with the neck of the combustion tube is also attached, after which one removes both the absorption train and the substance to be analysed, which lies on the copper block in the desiccator, into the neighbourhood of the actual combustion apparatus.

The calcium chloride tube is then attached to the neck of the combustion tube, and care is once more taken that all the connections are so adjusted that the glass portions are in immediate contact. The soda-lime tube is then connected with the Mariotte flask by connecting it with the calcium chloride tube which is, in turn, connected with the end of the rubber tube leading to the apparatus.

The rubber stopper is then removed from the combustion tube, and in order to have plenty of room, the long stand from which the U-tube with bubble-counter is suspended is pushed to the back ; the copper block is then carried in one hand to the open mouth of the combustion tube until its edge is in contact with it, whilst with the other hand the boat is held with the forceps and pushed into the open tube. It is then pushed so far forward with a clean glass rod that an empty space of about 10 to 15 mm. remains between the tube filling and the boat. The mouth of the tube is most easily closed by removing the tapered capillary from the rubber stopper, pushing this latter into the mouth of the tube, replacing the retort stand with the bubble-counter, and then pressing the tapered capillary into the rubber stopper.

The whole system may now be tested for serious leakages by turning the three-way cock through an angle of 45 degrees, and lowering the syphon of the Mariotte flask slightly. One then notes whether, after a few minutes, gas bubbles still rise inside the Mariotte flask. In this case, which seldom occurs, it is best to remove the weighed material from the tube in order to conserve it, and then to make the leaky joints good. It should be observed, however, that an approximate steadiness of the level of the liquid in the capillary is no proof that the rubber connections are thoroughly tight in a microanalytical sense.

One then notes whether the pressure regulator for the oxygen is properly filled and is in the correct position as already determined. The three-way cock is then turned into the proper position, and the syphon of the Mariotte flask is lowered, watching the bubble frequency meanwhile, until the number of bubbles per ten seconds corresponds with that previously determined.

The copper heater is then placed in position over the two constrictions in the side tube of the calcium chloride tube in order to prevent the condensation of any appreciable quantity of water at these points, and the soda-lime tube is covered over the whole length of its filling with a double layer of flannel soaked in cold water. The combustion of the material is then proceeded with as follows :—

The short adjustable roll of wire gauze round the combustion tube is so placed that its front edge just reaches to the handle of the boat, and it is then heated from below with the movable burner, the full flame of which is just free from luminosity. The heating of the tube is followed by a diminution of the bubble velocity in the bubble-counter. After some time this returns to the original frequency.

After some minutes melting, sublimation or carbonisation of the particles of material near the heated section will usually be observed. When these phenomena have completely ceased, the material is further heated by moving the gauze coil some millimetres over the boat. It is necessary to proceed slowly, as if large quantities of vapour are evolved, which is immediately indicated by complete stoppage of the gas current through the bubble-counter, these will strike back towards the rubber stopper and will necessarily lead to serious losses.

Every time that the burner and gauze are moved forward the frequency of the bubbles is somewhat diminished. It is then necessary to leave the burner stationary for so long, at least, that the original bubble frequency is restored ; therefore it is necessary to watch both the material to be analysed, and the bubble-counter

Substances which form on heating a nitrogenous charcoal which. is difficult to burn and which therefore cause considerable difficulty during macroanalysis, can easily be burnt completely in the microanalytical carbon and hydrogen determination ; by removing the burner completely after ignition for some time and allowing the boat to cool, the residual charcoal particles burn readily on reheating, usually with the production of a shower of sparks. When the whole of the material has finally been oxidised with the above-mentioned precautions and the movable burner has been moved up to the tube burner, the pressure regulator for the air, which has already been filled, is adjusted to a level 10 to 20 mm. lower than that of the oxygen pressure regulator ; air is then led into the combustion tube by turning the three-way cock, and from this period the water which flows from the Mariotte

flask is collected in a measuring cylinder. The empty portion of the tube is now once more heated with the movable burner, starting from a point about 70 mm. from the hinder rubber stopper. When 100 c.c. of water have been collected one may be sure that all the carbon dioxide and water vapour have been carried into the absorption tubes. In the case of substances containing much hydrogen, over 10 per cent., it is advisable to leave the calcium chloride tube, at any rate, in connection with the tube for a further five minutes; since lead peroxide asbestos has been used, this precaution has become superfluous. The time required from the commencement of the combustion up to this point is on the average twenty to twenty-five minutes.

If one is using good reliable rubber connections, it is possible to carry out the analysis without the Mariotte flask. In this case it is equally necessary to observe the precautions mentioned on p. 50 regarding the speed of the gas current. It is usually sufficient finally to pass an air current of 4 to 5 c.c. per minute through the tube for ten minutes. In the case of substances containing more than about 10 per cent. hydrogen, at least fifteen minutes is necessary.

Before removing the absorption tube, the outlet tube of the Mariotte flask is turned upwards, the rubber connection is removed from the end of the soda-lime tube, the heater is removed from the inlet of the calcium chloride tube and the moist flannel from the soda-lime tube. The combustion tube is then held with one hand, and the rubber connection with the calcium chloride tube is disconnected from its neck. The absorption tubes are then removed to the neighbourhood of the balance while still rigidly connected by the central rubber connection. They are then cleaned and wiped dry as already described and left to cool. The cooling period is used to remove the boat from the combustion tube by means of a platinum hook fixed into a glass rod; the boat is then either reweighed, if necessary, in order to determine any ash or residue, or it is immediately boiled with dilute nitric acid, ignited and weighed, so that it is ready to receive the material for the next analysis. After being cooled for the necessary fifteen minutes and weighed the absorption tubes are ready for the next analysis, as is also the combustion tube. In this manner it is possible with one apparatus to carry out successive analyses in an average time of about forty to fifty minutes.

Salts of alkalies and alkaline earths are covered, after being weighed into the boat, with sufficient potassium dichromate to

cover the point of a penknife. The potassium dichromate should be recrystallised three times and converted into a finely crystalline powder by rapid cooling. It should always be kept over sulphuric acid in a desiccator after being heated almost to its melting point, and if necessary, finely ground ; in such cases the heating of the boat should be carried out with particular care in order to avoid spirting of the molten potassium dichromate and consequent contamination of the tube.

If the combustion of such salts is often undertaken, it is advisable to use for the purpose an old combustion tube which is almost exhausted. The boat is best cleaned after such analyses by heating it with potassium sulphate and concentrated sulphuric acid in a platinum dish.

The analysis of salts of gold, platinum and silver is particularly simple, as the percentages of noble metals can readily be determined by reweighing the boat after combustion and appended to the results of the carbon and hydrogen determinations. Copper can also be determined in this way if the combustion is carried out very carefully, so that no metallic copper is separated in consequence of any deficiency of oxygen. If this is avoided, the copper remains in the boat entirely as cupric oxide, and can subsequently be removed completely by boiling with dilute nitric acid. If, however, at any period of the combustion, metallic copper is formed, there is danger of it alloying with the platinum of the boat, and in addition to the consequent low value for copper, the boat is permanently damaged. Iron in iron salts can be determined with much less risk, and is finally weighed as ferric oxide.

The Course of the Determination of Carbon and of Hydrogen with the use of a Mercury Gas-holder

For the sake of completeness, and also on account of its historic interest, and at the wish of my friend and colleague J. Herzig, of Vienna, I mention here that my first successful attempts to determine carbon and hydrogen in small quantities of organic substances were accomplished by collecting the combustion products, which still contained a certain quantity of unburnt material in consequence of too short a length of tube filling, in a mercury gas-holder from which they were once more passed through the heated combustion tube. This procedure is, when carefully considered, not quite comparable with the process of combustion of the same material with a more ample tube filling, for the combustion gases which are collected in the mercury gas-holder are quite

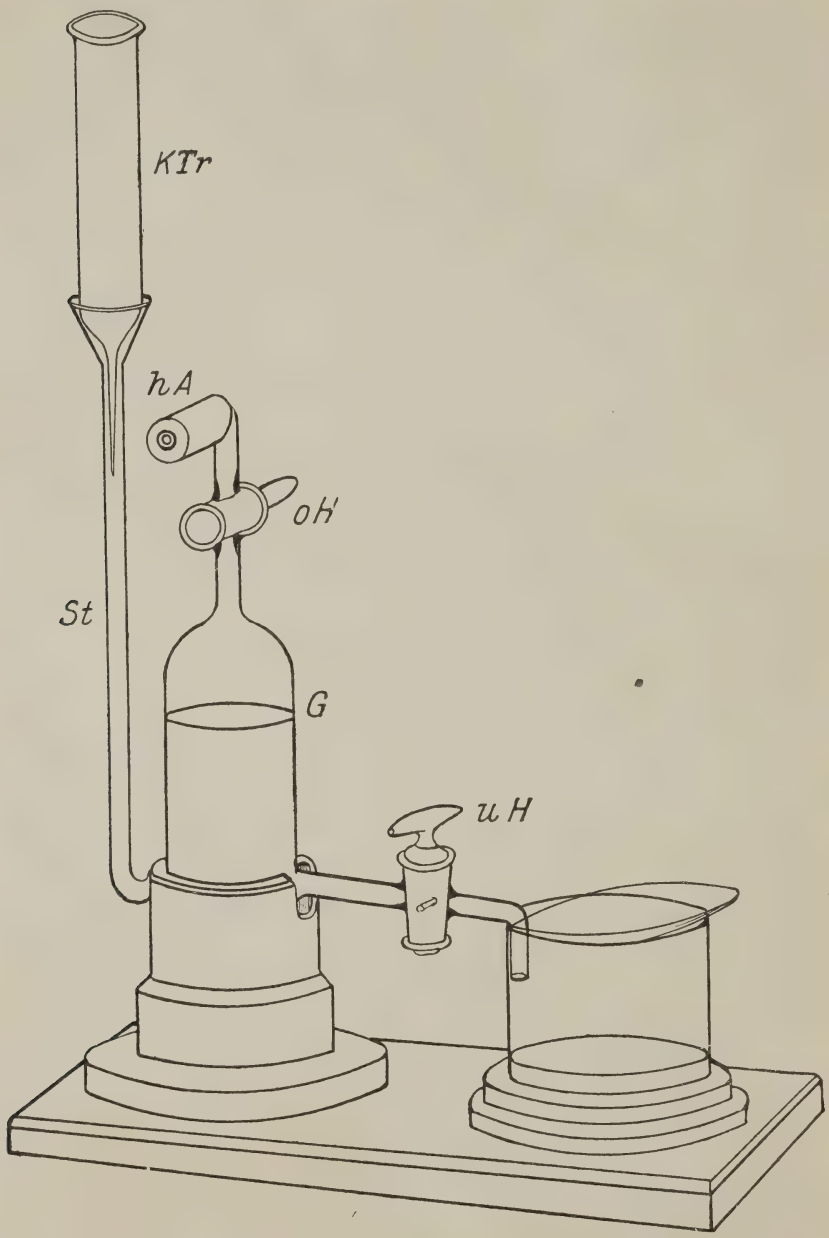

Fig. 17.—Mercury gas-holder. ($\frac{1}{2}$ actual size.) *G.* Gas-holder. *oH.* Upper cock. *uH.* Lower cock. *St.* Levelling tube. *hA.* Horizontal side tube. *KTr.* Capillary funnel.

free from carbon dioxide after passing the soda-lime apparatus for the first time, and it is conceivable that in quite exceptional

cases the removal of this product of the reaction is more favourable for the complete oxidation of gases which are combustible with difficulty, than an increase of the tube filling only. For this reason the mercury gas-holder (Fig. 17) and its manipulation will be shortly described here.

It consists of a cylindrical glass vessel of about 100 to 120 c.c. capacity, the upper narrow end of which carries a glass cock *oH*, and above this a horizontal connecting tube *hA* bent at a right angle, the diameter of which is preferably 3·5 mm. This cylinder has a rounded sealed bottom from which issues on the one side a glass cock *uH* for discharging the mercury, and opposite to this a vertical glass tube *St*, of 7 mm. diameter, and 200 mm. long, which has a funnel-shaped expansion at the upper end. This glass vessel is carried by a wooden base so arranged that the upper horizontal connecting tube is at the level of the neck of the combustion tube, *i.e.*, 215 mm. above the bench top, and that the mercury discharged from the lower cock can readily be collected in a glass dish with lip which is placed below it. There is also provided a capillary funnel *KTr*, which consists of a thick-walled glass tube about 100 mm. long and of 20 mm. diameter, which is drawn out below to such an extent that the filling of the gas-holder with mercury through this capillary funnel and the funnel-shaped expansion of the levelling tube requires at least twenty minutes.

The use of this mercury gas-holder can be described in a few words in addition to our previous description of the carbon and hydrogen determination. After introducing the material into the combustion tube and attaching the two absorption tubes, the mercury gas-holder, which has previously been filled with mercury, is attached to the absorption tubes by directly connecting the horizontal side-tube of the gas-holder to the end of the soda-lime tube by means of rubber tubing. The upper cock of the gas-holder is then opened and mercury allowed to escape drop by drop through the lower cock at a rate which does not exceed two drops per second. The pressure within the gas-holder and the absorption tubes can be continuously controlled by means of the levelling tube of the former. As has already been explained, it should never be greater than the atmospheric pressure, *i.e.*, the mercury level in the levelling tube should never be higher than that in the gas-holder itself. Moreover it should never drop more than 5 mm. during the combustion, this amount corresponding to a difference of head of about 60 mm. of water which is the amount which is

obtained with the Mariotte flask. The combustion of the material is carried out as usual. During the period of maximum absorption of carbon dioxide the pressure in the gas-holder drops rapidly, and it is necessary, in order to avoid an excessively reduced internal pressure, to close the outlet cock until the level in the pressure tube begins to rise again, after which the mercury is once more allowed to escape at a rate of two drops per second. When the combustion of the material is complete and the empty portion of the combustion tube is once more being heated with the movable burner, both cocks of the gas-holder are closed, and the U-tube with the bubble-counter, together with the rubber connecting tube, is removed from the thermometer tube which passes through the rubber stopper of the combustion tube. The gas-holder is then disconnected from the rubber tubing leading to the soda-lime tube. The gases enclosed in the gas-holder, which have already passed once through the absorption tubes, are then passed through the red-hot combustion tube a second time by connecting the gas-holder with the other end of the latter by means of the tapered thermometer tube in the rubber stopper.

By opening the upper cock of the gas-holder and pouring in mercury through the capillary funnel and pressure tube, the collected combustion gases are passed at a uniform rate through the red-hot combustion tube. When the gas-holder is filled with mercury up to the level of the upper cock, the original disposition of the apparatus is resumed, *i.e.*, one connects the U-tube with the bubble-counter with the tapered capillary funnel and the gas-holder with the soda-lime tube, after which the oxygen is replaced by a current of air, and mercury is allowed to escape from the gas-holder at a rate of two drops per second until this is empty. The absorption tubes are then disconnected and ultimately weighed.

IV. Gas-Volumetric Determination of Nitrogen in Small Amounts of Organic Material (Micro-Dumas Method)

In my paper on quantitative microelementary analysis of organic compounds, which has already repeatedly been referred to, I described in detail a method of determining nitrogen in which a volume of gas was obtained one-tenth of which had always to be deducted in order to obtain a correct nitrogen value. This excess in the volume of gas obtained was at first ascribed by me to the space taken up by the 50 per cent. caustic potash adhering to the walls of the microazotometer. Direct measurements which were carried out later showed that the volume taken up by the

50 per cent. caustic potash in tubes of equal dimensions to the measuring tube of the new precision microazotometer was only about 2 per cent. (1·7 per cent.) of the measured volume.

This ratio was determined as follows : Tubes of the dimensions already referred to and which were sealed at one end, were filled with water up to the top from a burette such as I use in carrying out the micro-Kjeldahl determination. The tube was then dried, filled with 50 per cent. caustic potash, then drained and again filled with water from the same burette. The difference of the two burette readings gave an average of 1·70 per cent. of the volume of the tube.

Dr. Brunner[1] who had studied my methods in Innsbruck, paid special attention to this correction and established gravimetrically that the volume diminution caused by 50 per cent. caustic potash was 1·50 per cent. He also found that on adding a correction for the alteration in vapour tension the correction of 2 per cent. of the value of the reading, which had been used by me for some time, was practically exact.

Eight per cent. of the volume correction for the gas which was evolved in the method of nitrogen determination already described by me, had at that time therefore to be described as "inevitable." A series of experiments gradually established the nature of this discrepancy and the reason for its constant ratio to the quantity of nitrogen in the material, and finally indicated the methods which were necessary in order to eliminate it completely, and thus to elaborate a method which was completely independent of an empirical correction such as had hitherto been used. I will therefore call the previously described micro-Dumas method "the empirical rapid method," and will denominate the newly elaborated method as the exact micro-Dumas method, but wish to make it clear that the rapid empirical method yields exact results in experienced hands.

When this rapid method was used for compounds free from nitrogen or for sugar charcoal, several milligrammes of the material yielded a volume of gas which did not reach the upper scale division of the microazotometer. If, however, the amount of the material was increased, and more particularly if the combustion was carried out very rapidly, considerable amounts of gas were obtained which burnt with a bluish flame and which showed with dilute blood solution the properties and reactions of a carbon monoxide-hæmoglobin solution. This result indicated that the

[1] Inaugural Dissertation, Freiburg-i.-B. (1914), pp. 38–40.

rapid method yielded nitrogen mixed with carbon monoxide. In order to confirm this result, nitrogenous substances were analysed according to the established rapid method, but the gases which were produced were not collected in the microazotometer as in ordinary analysis but over dilute blood solution. This also showed the reactions of carbon monoxide-hæmoglobin, the most serviceable of which is that with hydrogen sulphide solution. As I had found it necessary to deduct 10 per cent. from the gas volume in order to obtain the true nitrogen volume, and as, moreover, direct measurement had shown the influence of the caustic potash adhering to the walls to be about 2 per cent., it was clear that the unavoidable and hitherto unexplained 8 per cent. correction must be due to carbon monoxide, and possibly also to some other associated gas. It is not impossible that oxygen is also present, together with the carbon monoxide.

I also discovered that apart from too short a period of contact between the combustible gases and vapours and the red-hot tube filling, the increase of the gas volume obtained might also be partially due to the action of the reduced copper spiral, as is clear from the following experiment :—

A reduced copper spiral is introduced into a small combustion tube, and the air is replaced by carbon dioxide from a Kipp generator, during which process the copper spiral is heated in order to make certain that all adsorbed gases are removed. The spiral is then allowed to cool in the current of carbon dioxide. If the microazotometer is now attached to the combustion tube it will be observed that the rising bubbles of carbon dioxide disappear, apart from an almost invisible residue. As soon, however, as the burner is once more placed below the copper spiral the ascending bubbles become larger, and in this manner measurable quantities of gas can be collected if the duration of the experiment is sufficiently long. Clearly the phenomenon is due to a permanent disturbance of the equilibrium between carbon dioxide on the one hand and carbon monoxide and oxygen on the other, caused by the red-hot copper spiral. All copper spirals are not equally active in this respect. Apparently the phenomenon is mainly caused by certain impurities in the copper which vary in amount, perhaps by zinc.

This disturbance of equilibrium between carbon dioxide on the one hand and carbon monoxide and oxygen on the other remains unaffected whether the tube is charged with metallic copper only or whether any quantity of copper oxide is placed behind the

terminal copper spiral, *i.e.*, nearer to the point of entrance of the current of carbon dioxide. The results of the experiment are, however, quite different if a considerable quantity of copper oxide is placed in front of the mass of metallic copper in such a manner that the portion adjacent to the outlet for the current of carbon dioxide extends beyond the heating flames. This arrangement ensures a temperature fall at this point which causes the complete oxidation of the carbon monoxide which is formed. In this case, whether the tube is heated or not, minute bubbles only rise in the microazotometer which yield no measurable volume of gas even if the experiment lasts for half an hour. If one assumes that the diameter of the ascending gas bubbles is about 0·2 mm. (and if the Kipp generator is treated properly, much smaller bubbles are easily obtained), then the volume of such a bubble is about 0·004 cu. mm., *i.e.*, 250 such bubbles have a total volume of 1 cu. mm. = 0·001 c.c., and if we allow one such bubble to ascend per second, then a volume of 0·014 c.c. only will be obtained in one hour.

These phenomena had not entirely escaped the attention of former careful observers such as Ernst Ludwig and Guido Goldschmiedt, and these workers and others already recommended, when carrying out nitrogen determinations by the method of Dumas, the addition of an oxidised roll of copper gauze behind the reduced copper spiral. They observed that the latter was more or less reduced at each determination and that the values which were obtained were much more accurate, as the end of the analysis could be ascertained with absolute certainty, the bubbles being then as small as at the commencement of the analysis.

These results decided us definitely to change the position of the reduced copper to the middle of the tube, especially as a further consideration rendered this all the more imperative. It has now been established that during the combustion of organic substances by Dumas's method, nitrous oxide may also be obtained. This would not in itself affect the analytical result, as nitrous oxide takes up the same volume as the nitrogen which it contains. On the other hand, at very high temperatures, nitrous oxide is dissociated into nitrogen and oxygen, with the result that the volume is increased to one and a half times the volume of the original nitrous oxide, or of the nitrogen contained therein. It is clear that this dissociation will take place to the greatest extent in the hottest part of the combustion tube, and that at such temperatures metallic copper will absorb the oxygen which is produced most effectively. The

correctness of this view is proved, on the one hand, by the fact that I was able to convince myself by numerous experiments, which were at first not premeditated, but were confirmed by others carried out with deliberate purpose, that when metallic copper was used in too small an amount, or when insufficiently heated, that is when the time of contact between the gas current and the copper was too short, results were obtained when determining the nitrogen in various substances, more particularly in azobenzene, which showed nitrogen values up to 1 per cent. above the theoretical amount. If, on the other hand, larger quantities of copper were used at the hottest portion of the combustion tube, correct nitrogen values were immediately obtained, by deducting 2 per cent. from the nitrogen reading. A still more striking confirmation of the fact that the most correct procedure is to place a considerable quantity of metallic copper in the hottest zone of the combustion tube between two fairly long layers of copper oxide is the following :—

If the nitrogen is determined, in substances which are difficult to burn, by combustion when mixed with a considerable amount of potassium chlorate, in order completely to destroy the residual carbon, then in spite of the considerable amounts of free oxygen which are evolved, correct nitrogen values are obtained, as the large mass of red-hot copper constitutes an impassable barrier for the oxygen.

The fact must be noted here that the phenomenon which has just been described may also occur if a tube filled with copper oxide only is alternately heated and cooled, in which case rather larger bubbles will always be observed to rise in the azotometer after heating and will again become smaller after cooling, until they dissolve in the caustic potash leaving a scarcely visible trace behind. This occurrence can only be explained by assuming that on heating a perceptible amount of dissociation of the carbon dioxide occurs into carbon monoxide and oxygen, and that at the gas velocity which is used these gases do not recombine, as they are cooled too rapidly.

Similar observations have previously been made by careful observers when using Dumas's original method. It is well known that if the tubes are too strongly ignited when using Dumas's old method, blank tests yield an indefinite quantity of undissolved gas which increases with the duration of the experiment and the velocity of the gas current.

These observations led to the precaution of allowing that

portion of the tube which was filled with copper oxide to project for some centimetres beyond the burners, in order to ensure a gradual fall in temperature of the gases and thus to facilitate the oxidation of any carbon monoxide to the dioxide.

The observations above referred to led to the adoption of methods of filling and heating the tube which rendered it possible to liberate the nitrogen contained in an organic substance free from impurities, and to recognise the end of the reaction with certainty. It is very remarkable that a number of sources of error which had been observed by few in the course of macro-analysis, were only definitely recognised on studying the micro-analytical methods. The principles thus discovered were afterwards applied to macroanalysis with complete success.

Apart from the filling of the tube, the rate at which the bubbles rise in the azotometer is very important. If the nitrogen is evolved at such a rate that the bubbles rise not more than once in two seconds, correct nitrogen values are obtained ; that is, the final gas volume, after deducting 2 per cent. (which is essentially due to the volume taken up by the caustic potash adhering to the walls of the azotometer), corresponds with the nitrogen in the organic material undergoing analysis. If, however, the bubbles rise at a greater rate than that mentioned, that is, if more than one bubble rises per two seconds, then even if the tube is correctly filled, nitrogen values are obtained which are too high ; that is, after deducting 2 per cent. of the reading a volume is obtained which does not consist of nitrogen only, but of a mixture with other gases ; these evidently consist of carbon monoxide which has not been oxidised in the time during which it was in contact with the glowing tube filling in consequence of the rapidity of the gas current. It will be clear from the above that this error would be still greater if the terminal portion of the filling consisted of metallic copper.

We are now in a position to explain the constant value of the empirically determined correction of 10 per cent. when using the old rapid method. The impurities which are then obtained in the gas depend on conditions favouring their production, more particularly on the terminal position of the copper spiral, and also on the period of contact of the products of combustion with the glowing tube filling in general. This period depends not only on the length and cross-section of the tube, but also on the rate at which the gas is evolved. This in turn depends on the amount of nitrogen introduced into the tube in the material, for the speed of

evolution of the gas is gauged by the rate at which it rises in the azotometer after being freed from carbon dioxide. This explains the empirically determined correction of 10 per cent. which has already been mentioned in connection with the older rapid method, and the strict proportionality of the impurities to the total nitrogen for all substances investigated and quantities used for analysis under the conditions then employed.

We have already mentioned that when using the old method of Dumas any quantity of gas could be obtained during the blank tests by strong heating and increasing the rate of evolution of the gas. In spite of the essentially unfavourable tube-filling in the Dumas nitrogen determination, satisfactory nitrogen values were generally obtainable, although my microanalytical experiments showed the essential importance of proper tube-filling and adequate time of contact of the evolved gas with the latter ; the explanation of this fact appears to me to lie essentially in the difference in capacity between the old combustion tube and that used in microanalytical combustions. The use of the ordinary combustion tube ensures a considerable time of contact of the gaseous products of combustion with the tube-filling without special precautions. In addition we have the fact that in accordance with the kinetic theory of gases, the molecules of a gas of low molecular weight diffuse very much more rapidly than those of a gas of high molecular weight. In our case the carbon monoxide and nitrogen will both travel more rapidly than the carbon dioxide, and if the gas velocity exceeds a certain upper limit, the escape of unburnt carbon monoxide will be inevitable. It is thus clear that this phenomenon will be much more strongly developed in the short microanalytical combustion tube of smaller capacity than in the long roomy tube which is used in macroanalysis.

It was found possible by the end of 1912 to elucidate and prevent, as already described, all those sources of error which influence the volume of nitrogen which is isolated in the combustion of organic substances.

By using a tube-filling which prevented the production of an admixture of foreign gases, and by strict observance of the necessary minimum time of contact of the organic vapours to be burnt with the heated tube-filling, a method was developed which liberated free nitrogen only from organic substances and enabled it to be measured with accuracy and independently of compensating errors. This is shown by the fact that since the date already mentioned a large number of compounds of the most

various classes have been analysed by myself and by colleagues with entirely satisfactory results. A further proof of the general applicability of the method is shown by the fact that from the end of 1912 onwards, in spite of thousands of analyses, there has been no desire, and not the slightest necessity, for any alteration of the method.

Dubsky alone has found it desirable to modify the conditions laboriously studied by me, and to return to the terminal copper spiral in order to establish a "simplified or semi-microelementary analysis" by increasing the amount of material. He attempts to query the results of my exhaustive experiments with the question, "Is it possible to recognise the end of the combustion by means of the gas bubbles if the reduced copper spiral decomposes carbon dioxide with formation of the monoxide?" [1] He overlooks the fact that the conditions governing the whole course of the analysis are more important in obtaining correct results than those at the end. I am, therefore, content to leave it to my colleagues to decide whether my precautions are unnecessary or whether on the contrary Dubsky's modifications are a retrograde step.

A. Schoeller's comment is as follows: "The above remarks also apply to Dubsky's "simplifications" of the nitrogen determination involving larger quantities of material and a terminal spiral." He also writes: "It appears to me doubtful whether the method is in fact simpler and easier than the well-established methods of Pregl."

In describing the method I will first consider the individual portions of the apparatus, following the direction of the current of carbon dioxide. Therefore, we will first consider the production of pure carbon dioxide free from air, then the delivery of this to the combustion tube, the combustion tube itself and finally the collection and measurement of the nitrogen obtained in the precision microazotometer. We will then finally describe the course of the analysis.

Kipp Generator for Production of Carbon Dioxide

As the carbon dioxide for the volumetric determination of nitrogen must be extremely pure in order that it may be absorbed by 50 per cent. caustic potash with a scarcely visible residue (micro-bubbles), attention must be paid to the filling of the Kipp generator (Fig. 18, p. 81).

In order to remove air from the apparatus as rapidly as possible

[1] *Berichte*, (1917), **50**, p. 1710.

on starting, a hook-shaped glass tube h is attached by a short piece of rubber tubing to the inner end of the glass cock of the middle bulb of the apparatus by means of a rubber stopper. By this means the gas is expelled from the highest point of the middle bulb. The middle bulb is completely filled with medium-sized pieces of white marble. Each piece is etched with a little hydrochloric acid and washed under the tap. Broken glass, short glass rods or similar materials are better than a leather or rubber disc for preventing the marble from dropping into the lower bulb. The apparatus is filled through the upper bulb with a mixture of equal parts of fuming hydrochloric acid and tap-water to such an extent that the lower bulb and about half or one-third of the upper bulb are filled. If the cock H_1 is then opened, air is expelled from the middle bulb and carbon dioxide is liberated by the acid which enters. The gas which is then obtained from the apparatus by no means conforms to the high degree of purity which is necessary for the nitrogen determination, for the hydrochloric acid still contains a large amount of air in solution which mixes with the carbon dioxide. Two or three lumps of marble of the size of filberts are therefore dropped into the upper bulb ; these lodge in the central tube and develop considerable quantities of carbon dioxide which completely remove the air from the hydrochloric acid in the upper bulb. The process is hastened by mixing the whole volume of acid by repeatedly opening and closing the cock. A freshly assembled Kipp generator, which has been treated as just described, does not as a rule deliver gas of the necessary high degree of purity. It is therefore best to allow it to stand for two or three days, after which fresh lumps of marble are thrown into the upper bulb in order to repeat the de-aëration. It is evident that new generators hold large amounts of air, not only on the inner surfaces of the glass, but also absorbed in the rubber stoppers, and this is only liberated after being exposed for days to an atmosphere of carbon dioxide. After such an interval only will it be found that the carbon dioxide is completely absorbed by caustic potash so that the bubbles vanish, leaving a scarcely perceptible trace. In the case of apparatus which has already been used, and is immediately refilled after being completely cleaned, this delay of several days is not necessary before use. If one estimates the diameter of the micro-bubbles which are formed at one-tenth of a millimetre, simple calculation shows that 2,000 such bubbles only measure 0·001 c.c. On the assumption that the diameter is 0·2 mm., 250 such bubbles have a volume of 0·001 c.c.

If the acid in the apparatus has become weakened by use, it is advisable to empty it in such a manner that no air enters the

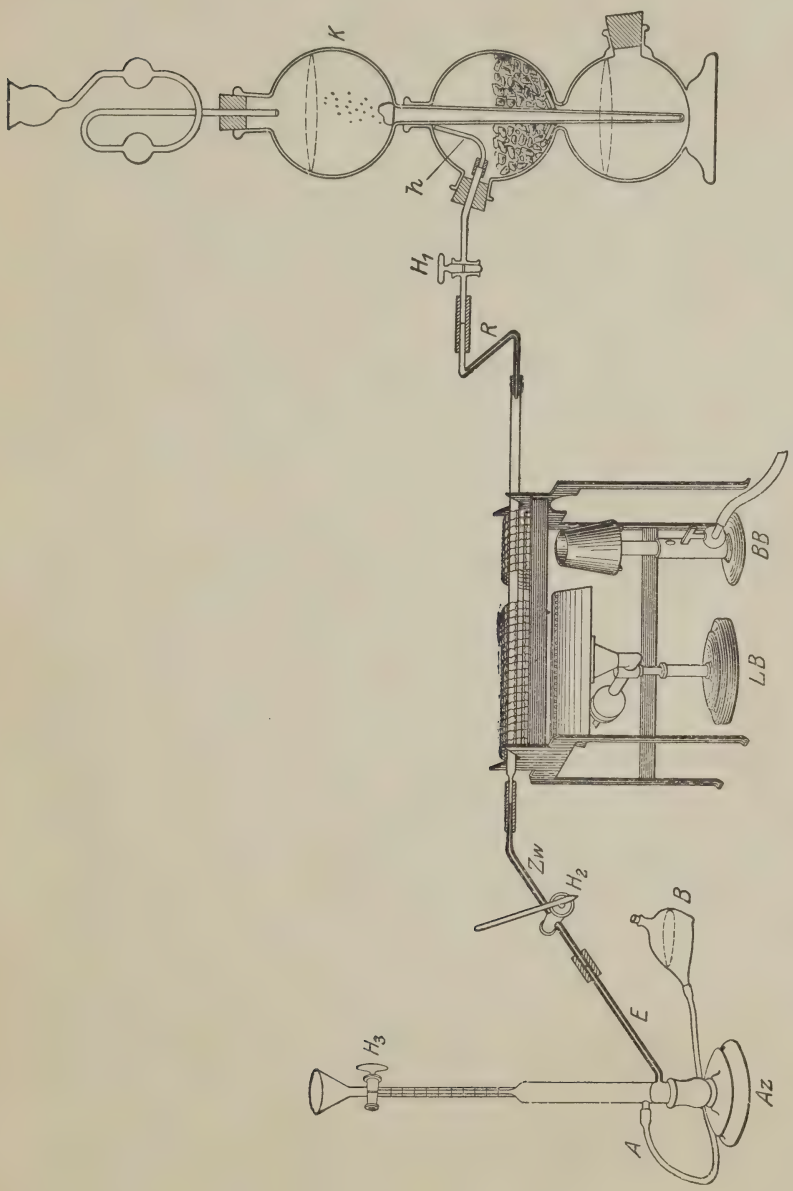

FIG. 18.—Apparatus for volumetric nitrogen determination. (½ actual size.)

K. Kipp generator with bent tube *h* and cock H_1. *R.* Bayonet-shaped tube connecting Kipp generator with combustion tube. *LB.* Tube burner. *BB.* Movable burner. *Zw.* Connecting-piece with cock H_2, with lengthened handle. *Az.* Precision azotometer with cock H_3. *E.* Delivery tube. *A.* Side tube for rubber tubing. *B.* Levelling vessel.

middle bulb. This is done either by syphoning it from the upper bulb of the apparatus after the acid has risen into the same, or by running it away through a glass cock fixed into the tubulus of the

lower bulb. The rejected acid is replaced by adding the same volume of concentrated pure acid, which is diluted by the spent acid which remains. The high proportion of calcium chloride in the latter is very advantageous, as it lowers the coefficient of absorption of the mixture for the constituents of air, compared with that of a mixture of acid and pure water.

The carbon dioxide is delivered to the combustion tube through a glass connection *R* of thermometer tubing, at the end of which a glass tube, of the same diameter as the tube at the end of the Kipp generator, is attached. The other end of tube *R* is connected by means of a closely-fitting piece of rubber tubing with a tapered thermometer capillary in order to render the apparatus handier and less easily breakable. This tapered capillary is fitted into the mouth of the combustion tube by means of a rubber stopper. The thermometer tube itself is either bent into the shape of a bayonet, so that the Kipp generator lies further away from the experimenter and from the edge of the bench, or it may be bent twice at right angles in the same direction so that the Kipp generator lies behind the combustion stand and takes up a lesser length of the bench. The widened glass attachment of the thermometer tube is connected to the horizontal glass cock of the Kipp generator by means of a well-fitted rubber tube, moistened with a little glycerine within, in such a manner that there is glass to glass contact. This tube is then wrapped in several layers of thick paper in order to render it more rigid. In order to avoid subsequent bending of the combustion tube, the other end of the thermometer tube to which the tapered capillary is attached is provided with a wire support in the shape of an inverted V which holds it at a height of 21 cm. above the bench top.

In the *Zeitschrift für angewandte Chemie*, of November 25th, 1921, No. 94, p. 586, Schöller describes a micro-Kipp generator which he has found to be exceptionally efficient. So far I have unfortunately not been able to try it, although I wish to mention it. I have therefore described in detail the use of the ordinary Kipp generator which is generally available in laboratories, in conjunction with the micro-Dumas method.

I must also mention here an unusual but disturbing source of error. Tubing through which gaseous ammonia has been passed is useless for the tube connections, as its use results in nitrogen values which are too high for a long period. Finally, I must mention an unsoundness which has developed in course of time in rubber connections between the Kipp generator and the com-

bustion tube. Such leakages are often the cause of results which are too high in consequence of the admission of small quantities of air. Last year excessive nitrogen values were frequently obtained in my laboratory, and as most of the analyses were concerned with arsenical compounds, it was supposed that the efficiency of the tube filling might have been affected by the combustion of such compounds. I was able to convince myself later that the trouble was substantially due to unsoundness of the rubber tubing caused by aging, and I was able to show that the gas-volumetric method of determining nitrogen, as described by me, gives absolutely correct values for substances containing arsenic.

The Filling and Preparation of the Combustion Tube for the Gas-Volumetric Determination of Nitrogen

A combustion tube 400 mm. long, with attached neck, such as has already been described in connection with the determination of carbon and hydrogen, is used. Into this an ignited wad of asbestos is introduced at the wide end, and is pressed with moderate force into the neck with the help of a glass rod. Some wire-form copper oxide is then introduced, the longer particles of which have been broken by gentle crushing in a mortar ; this is added to a length of 130 mm., and in order to hold this permanent filling in position a further asbestos plug is introduced and gently compressed. The shorter coil of wire gauze, 40 mm. long, is then passed over the filled tube, which is placed on the combustion stand ; a section of the copper oxide about 40 cm. long, starting at the last-mentioned asbestos plug, is then slowly reduced step by step with the help of the burner in a current of hydrogen which has previously been washed with acid permanganate solution, and introduced through the wide opening of the tube ; the whole is then allowed to cool in a current of hydrogen.

The experience of the last few years has led me to the following conclusions with regard to the copper oxide and reduced copper which is used. Wire-form or finely granulated copper oxide is equally efficient, but it is important that the copper oxide surface available for oxidation should be as large as possible and that the individual grains, whether wire-form or granulated, should consist of copper oxide throughout. The use of copper oxide, obtained by oxidising short lengths of copper wire for filling the combustion tube for the nitrogen determination cannot therefore be permitted, as ignition, even though for a considerable time, only

oxidises the outer layer of the wire, and even this oxidised layer is liable to fall away and lay bare unoxidised copper. For the determination of carbon and hydrogen a tube charged with metallic copper, either in wire-form or in the form of gauze rolls, gives excellent results, and was often used by me, but in that case the filling is constantly ignited in a stream of oxygen ; such a filling is unsuitable for the determination of nitrogen, as copper oxide is present in the surface layer in such small quantity that it is soon reduced by the combustible vapours of the substance being analysed, in the absence of any oxidising agent, and thus leads to nitrogen values which are too high.

I find it convenient, more particularly if large quantities are used, to reduce a considerable quantity of either wire-form or finely granulated copper oxide at one time by ignition in a current of hydrogen. The product can be kept indefinitely, and at need it can be used for filling the tube without further precaution.

It is advisable, before using the tube with its permanent filling for the first time, to preheat it throughout in a current of pure carbon dioxide, in which it is then allowed to cool. When not in use it is left on the combustion stand, still connected with the Kipp generator and closed by the connecting piece leading to the azotometer, so that it remains filled with carbon dioxide under pressure. By using this precaution, it is possible to carry out several hundred analyses without any further reduction of the reduced portion. By always keeping the tube filled with carbon dioxide under pressure the further advantage is gained that air is more quickly and completely displaced before analysis, and for this reason I favour the production of carbon dioxide in a Kipp generator rather than by heating sodium bicarbonate ; the latter has the further disadvantage that volatile substances undergo considerable loss on passing over them a current of warm carbon dioxide and that the bicarbonate tube would necessitate another burner.

The permanent filling is followed by a filling which is renewed for each analysis by introducing wire-form copper oxide from the stock bottle, by scooping it out with the open end of the combustion tube, until a layer is obtained about 90 to 100 mm. long. The carefully ignited wire-form copper oxide is best kept in a thick-walled test-tube of about 30 mm. diameter which is perfectly closed with a cork. Ignited, finely powdered, or preferably, scaly copper oxide is kept in another similar tube. A small amount of the latter preparation is poured on the top of the coarse copper

oxide, to a length of a few millimetres, in order to prevent any particles of the material to be analysed from falling through into the coarse filling, and thus undergoing premature combustion.

The introduction of the weighed material, which is covered and intimately mixed with copper oxide in a well-corked mixing tube, is effected with the help of a filling funnel (Fig. 19). This is prepared by drawing out an ordinary test tube in the middle, so that the drawn-out portion is 5 mm. in diameter and about 60 mm. long. The funnel is inserted into the combustion tube, and the mixture of copper oxide and material to be analysed is poured in from the mixing tube after removing the cork by carefully turning it with constant tapping. In this way adherence of the mixture to the cork is avoided. In order to remove the remaining material from the mixing tube, it is filled from the stock tube with about half the previously used amount of finely-divided copper oxide, closed again with the cork, and well shaken, so that any adhering material is thoroughly mixed. The tube contents are then poured into the combustion tube, and the process repeated. By means of these operations, a layer of fine copper oxide of about 40 mm. in all, is formed in the combustion tube. After removing the filling funnel, a further layer of about 40 to 50 mm. of coarse copper oxide is added. That portion of the combustion tube adjacent to the neck is then covered with a suitable roll of wire gauze about 150 mm. long, whilst the other end is covered with a similar roll about 40 mm. long, and the tube is placed on the combustion stand ; the open end is then connected with the delivery tube from the Kipp generator by means of a perforated rubber stopper, and the neck is connected with the connecting-piece in the precision microazotometer by means of a sound thick-walled rubber tube.

FIG. 19.—Filling funnel. ($\frac{1}{2}$ actual size.)

The Precision Microazotometer

The microazotometer as originally made, and as described in my previous publication, to which I have often referred, showed various disadvantages and led to occasional errors, and I therefore induced the firm of Wagner & Munz, of Munich, to supply a modified form of great accuracy. A material improvement on the former apparatus has been attained by placing the measuring tube below the cock, H_3, of the azotometer. The apparatus is of

such dimensions that 1 c.c. occupies a length of 95 to 105 mm. One-third of the circumference of the tube is covered by a sealed-in strip of ground glass, which serves as a background, enabling the graduations to be read with extreme sharpness. The zero point of the scale is at the cock. The first scale division is about 0·03 to 0·05 c.c., and the end of the scale is about 1·2 c.c. A few azotometers have a scale up to 1·5 c.c. These latter, of course, have a longer measuring tube, and are therefore less convenient. The spaces between the divisions correspond to 0·01 c.c., and it is easy to estimate the volume to within 0·001 c.c. = 1 cu. mm., particularly with the help of a reading lens as supplied by Köhler, of Leipzig. The scale divisions take up three-quarters of the circumference of the tube. This arrangement enables one to read the volume without any error or parallax by so placing the lens that the scale divisions coincide on both sides. The preparation of the scale is of great importance, and it is carried out by reversing the azotometer, closing the cock, delivering into the measuring tube volumes of mercury of 0·05, 0·1, 0·2, 0·3, 0·5, 0·8, 1·1, 1·2 c.c., and then noting the highest point on the convex mercury meniscus. This method of preparing the scale depends on the assumption which is not theoretically accurate, but practically permissible, that the convex mercury meniscus in the reversed azotometer corresponds in position with the concave meniscus of 50 per cent. caustic potash solution in contact with the glass walls. At my request each instrument is graduated in this manner, tested and provided with a certificate by the Bureau of Standards (Austria). The technical production of the precision microazotometer was soon so perfected that on looking through the test certificates, I found that a large number were either entirely free from error, or showed at most deviations of 1 to 2 cu. mm. at individual points. Each precision microazotometer is provided with a certificate, a suitable wooden stand, and a connecting piece, *Zw*, with cock, H_2, by means of which it is connected with the neck of the combustion tube. Although a rubber connection could have been avoided by placing this latter cock on the gas inlet tube of the azotometer, this course was not taken as the apparatus would then have been too breakable, particularly as the cock, H_2, is provided with a long handle in order to enable it to be adjusted more easily.

Before being filled, the precision microazotometer is cleaned by rinsing it repeatedly with sulphuric-chromic acid and water, and is then allowed to drain in the reversed position. The

levelling vessel, *B*, which has also been cleaned and dried, is then connected with the side tube of the azotometer by means of rubber tubing. It is advisable to bind both ends of this tubing with wire. Pure mercury is added through the levelling vessel until the level is midway between the inlet tube, *E*, and the upper side tube for the attachment of the rubber tube, *A*.

Before introducing the 50 per cent. caustic potash solution, the cock, H_3, is carefully lubricated with a little vaseline. No other lubricating medium must be used as otherwise the 50 per cent. caustic potash begins to foam after a short time. Sufficient of this latter is added to fill the measuring tube, and about one-third of the pressure vessel. It occasionally happens that with azotometers, which have been filled with perfectly pure mercury and pure 50 per cent. caustic potash solution, when first used, the rising gas bubbles are held at the boundary between the mercury and the potash solution, and only rise after considerable shaking. This occurrence, however, ceases after the first few determinations, when sufficient finely-divided copper oxide dust has collected at the boundary between the two liquids.

Fifty per cent. Caustic Potash Solution

It is absolutely necessary, in order to read the gas volumes by the level of the 50 per cent. caustic potash in the precision micro-azotometer, that the surface should be absolutely free from foam. As the solution obtained from the best commercial caustic potash did not fulfil these conditions, a process had to be found of obtaining a solution of the necessary qualities. 200 grains of potassium hydroxide (Merck) in sticks are dissolved in 200 c.c. of water, and 5 gm. of finely-powdered barium hydroxide are added. After shaking, the liquid is allowed to stand for fifteen minutes, in order to allow the separated barium carbonate to settle, and is then filtered through a Buchner funnel, the filtering plate of which has been covered with Gooch crucible asbestos ; the first filtrates are returned to the funnel until a perfectly clear liquid is obtained. The caustic potash so obtained is stored in bottles with rubber stoppers. The foaming of the caustic potash solution can equally well be removed by addition of barium chloride as described in my first communication, but I now prefer treatment with barium hydroxide as a liquid free from halogen is then obtained.

Preparation of the Material for the Determination of Nitrogen as Gas

The material in which nitrogen is to be determined is first weighed in weighing tubes (Figs. 20, A and B), which are prepared by drawing out test-tubes in the flame. They are 30 to 35 mm. long and slightly tapered. The open end is about 4 mm., and the closed end about 2 to 3 mm. wide. When weighing hygroscopic substances, a stopper which fits into the conical mouth of the weighing bottle is prepared from a portion of the drawn-out tube; the outer end of this stopper is drawn out to a narrow handle. In order to be able to handle the tube without appreciable warming, it is fitted into a spiral of thin aluminium wire, the free end of which is shaped into a fairly large lateral loop to serve as a handle. Such a spiral is best prepared by winding thin aluminium wire two or three times round a glass rod of about 2 mm. diameter in such a manner that the ends are of unequal length and bent at right angles after finally being crossed. The longer end is bent upwards at right angles at about 4 mm. from the point where the ends cross, and the length of the shorter end and the place at which it is bent are so chosen that the weighing tube, when placed in the three-fold loop and laid down, only touches the supporting surface at three points, namely at the sealed end, at the point of the short end of the wire, and at the angle of the longer bent end of the wire.

Fig. 20A.—Weighing tube.

Fig. 20B.—Weighing tube with glass stopper and aluminium holder. (Actual size.)

It is advisable to note the weight of such a weighing tube in order to be able at once to estimate the amount of weighed material on the balance. Usually 2 to 4 mgm. of material are used for a nitrogen determination, but we wish to emphasise that perfectly accurate results can be obtained with much smaller amounts. The smallest quantity used by me for analysis was

0·08 gm. of veronal, with which I obtained an accurate result. The tube filled with the material is weighed to an accuracy of three decimal places, removed from the balance by holding the handle with two fingers of the left hand, and is then seized with the right hand which is covered with a piece of clean gauze. The material is then emptied from the weighing tube into the mixing tube, which is a small test tube about 70 mm. long and 10 mm. wide ; the mixing tube is also held obliquely with the left hand, with the mouth upwards, whilst with the right hand the weighing tube wrapped in the gauze is placed horizontally, so that its mouth is above the mouth of the mixing tube. The contents of the weighing tube are transferred to the mixing tube by gentle tapping, the weighing tube is then returned to a vertical position, and removed to the balance by seizing it by the handle with the left hand, after which a second weighing is carried out with an accuracy of three decimal places. If these operations have taken some time, it is advisable to leave the weighing tube a few minutes in the balance to cool, for the heat capacity of glass is so high that it is often warmed in spite of the gauze. The amount of material in the mixing tube can now be calculated from the difference between the two weighings. The substance is then covered with finely-powdered copper oxide to a height of 10 mm., and the tube closed with a tightly-fitting cork, free from pores and shaken. These mixing tubes are rinsed with nothing but fine copper oxide, and they are therefore immediately ready for further use after each weighing.

It should be added that in order to obtain correct values, substances which do not melt, or which are combustible with difficulty, must be finely divided and mixed with much finely-divided copper oxide, unless it is considered preferable to use a method of combustion with potassium chlorate, which will be described later.

The Course of Analysis

After introducing the substances the barrel of the cock, H_2 (Fig. 18), is removed from the connecting-piece between the neck of the tube and the azotometer, and a current of carbon dioxide is passed through the tube for some minutes. Simultaneously, the front portion of the tube, which is covered by the long roll of wire gauze, is carefully heated with the lowered flame of the tube burner, and the temperature raised to a dull red heat. If the Kipp generator has previously been properly handled, on now testing the gas passing through the tube, minute bubbles

(micro-bubbles) will be obtained after two minutes. For the purpose of this test the barrel of the cock, H_2, is replaced and so adjusted that one or two bubbles rise per second. After lifting the levelling vessel, B, to a height above that of the open cock, H_3, of the azotometer, the apparatus is filled with caustic potash solution up to the funnel and the cock closed. On lowering the levelling vessel, bubbles then rise, one by one, and the diameter of these when observed with the lens should not be more than 0·2 divisions of the scale. Nothing is gained by passing carbon dioxide for a longer time, and on the contrary this is inadmissible in the case of substances which are appreciably volatile, as in that case vapours of the material would be prematurely burnt and the nitrogen values obtained would therefore be too low. Such results were frequently observed by us when analysing nitrosodimethylaniline and dinitrotrichlorbenzene. Correct values could only be obtained regularly with these materials by driving the air out of the tube in a current of carbon dioxide for a period no longer than that necessary to produce bubbles of the necessary small size.

Apart from observation with the lens, one can decide whether micro-bubbles of sufficiently small size are being obtained by the very much lower speed at which they rise in the measuring tube of the azotometer, compared with the speed of ascent of large bubbles. When sufficiently small, they rise slowly and at short intervals, one after another, in a manner which is sufficiently peculiar to enable an experienced eye to judge without the lens whether the air has been completely displaced.

After the tube and gas are free from air, as determined in the manner indicated above, cock H_1 of the Kipp generator is closed, and the cock of the connecting-piece, H_2, is fully opened. The small roll of wire gauze is moved to the boundary between the empty part of the tube and the filling of wire-form copper oxide, and the movable burner is placed below it so that the portion of the tube covered by the short roll of gauze is just within range of the burner which is turned fully on and is just non-luminous. As flames in this condition flicker very easily, the movable burner should always be protected by a chimney. The heating of the tube at once gives rise to renewed evolution of gas bubbles into the azotometer which slackens after some time if the position of the burner is not changed. This interval is used to open the upper cock, H_3, of the azotometer with the levelling vessel, B, raised so that any turbidity in the caustic potash which has been carried up by the rising micro-bubbles may finally be removed. After closing

the cock and laying the vessel, *B*, on the bench, the movable burner, *BB*, is moved slightly in the direction of the filling with fine copper oxide, and is left at that point until the evolution of gas bubbles once more slackens. The gauze roll and burner are then moved forward again, always avoiding entry of bubbles into the azotometer at a rate of more than one per two seconds. It is easy to avoid evolution at a greater rate than this, firstly, by only moving the burner a few millimetres at a time when in the neighbourhood of the material to be analysed, and, secondly, by never moving it until the rate of evolution of the bubbles is much smaller than this. When the whole section filled with the material to be analysed has been traversed by the burner in the manner described, and the bubble-frequency begins to diminish on moving the burner further along the tube, it can be moved forward more rapidly until it is immediately behind the tube burner *LB*. The cock of the connecting-piece, H_2, is then closed, carefully pressing its barrel inwards for safety, the cock, H_1, of the Kipp generator is opened and the cock, H_2, is then very carefully adjusted with the help of the long handle, with which it is furnished, so that not more than one bubble rises per two seconds, a lesser speed being maintained by preference. This adjustment is considerably facilitated by cutting shallow channels in the barrel of the cock with a glass-cutter, which extend about 4 mm. round its circumference at both ends of the bore. It is necessary to use as little lubricant as possible for the cock in order not to fill up these channels. After several determinations the whole connecting-piece and cock must be cleaned and rinsed with alcohol and dried on the pump, particularly if the substances to be analysed contain much hydrogen. This is necessary in order to prevent the channels from being filled up by water produced during the analysis. This adjustment of the cock is probably the only feature of the volumetric determination of nitrogen which requires a little practice, as it must be so carried out as to ensure that the above-mentioned velocity of the gas current is not exceeded even for a few seconds. Should this occur, higher nitrogen values may be obtained than correspond to the theory. The period during which the gas is expelled is utilised by once more igniting the whole length of the tube with the movable burner. In doing this, it is of course sufficient to place the burner at about four different points successively within the section to be ignited, which extends from the filling nearest the point of entry of the carbon dioxide up to the section heated by the tube burner.

After some minutes, it will be noticed that the gas bubbles rising in the azotometer suddenly begin to diminish, and gradually acquire the size which we described as micro-bubbles as at the beginning of the determination. It makes no difference, once this condition has set in, whether the operation is stopped at once or after a period of a few minutes, for, as has been shown by calculation, the volume of the micro-bubbles is so small that on allowing them to continue for a further period, the total volume of gas in the azotometer is not appreciably effected.

At this point the determination has lasted for about twenty to twenty-five minutes ; to complete the analysis the cock, H_2, of the connecting-piece is closed, the levelling vessel is raised to a height of a few centimetres above the bench top as a precaution (we always hold it in the hand which holds the lower portion of the azotometer) in order to prevent air entering the azotometer, and with the other hand the rubber connection between the connecting-piece and the inlet tube of the azotometer is disconnected. The disconnected azotometer is placed on one side, and its gas contents are brought to approximately atmospheric pressure by placing the levelling vessel on an adjacent stand. This can also conveniently be used for supporting the thermometer horizontally, so that its bulb touches the gas-filled portion of the measuring tube of the azotometer.

Temperature equilibrium is established in ten to fifteen minutes, and readings are taken of the temperature (to within half a degree) and of the gas volume in the azotometer. The latter reading is taken by adjusting the lens to the approximate height of the meniscus of the caustic potash solution, holding the azotometer with the right hand by means of the funnel and raising the levelling vessel in the left hand until the liquid in the latter is at the same height as the meniscus ; levelling vessel and azotometer are then lifted until the reading can be taken with the lens. Some will possibly find it more convenient to take the reading whilst the azotometer remains on the bench in the wooden stand. When reading the volume, parallax should be avoided by so adjusting the lens that the scale divisions adjacent to the meniscus coincide with their continuation at the back of the tube, and the position of the lowest point of the meniscus should be estimated to within one-tenth of a scale division so that the reading is taken with an accuracy of 0·001 c.c. Finally, it is necessary to avoid holding the measuring tube with the hand or placing it near a lighting unit

during the reading, as the consequent warming would lead to an expansion of the gas in a comparatively short time.

It is to be noted that after repeated use the caustic potash solution sometimes leaks through the closed glass cock of the azotometer through deficient lubrication, and enters the upper portion of the measuring-tube. The solution remains hanging at the top, causing a positive error, which may amount to several hundredths of a cubic centimetre. Any such volume of hanging liquid can be forced back into the funnel without the slightest loss of gas by raising the levelling vessel above the level of the solution in the funnel and carefully opening the cock.

In order to assure himself of the constancy of the result, the beginner should not neglect to repeat the reading of the gas volume obtained in a few analyses after an interval of several hours, or after allowing it to stand overnight ; he will be surprised to find the result to agree in all cases within a few hundredths per cent., after due correction for the altered temperature and pressure.

As already mentioned, 2 per cent. of the volume of nitrogen found must be deducted in order to allow for the space taken up by the 50 per cent. caustic potash solution. This calculation is most easily carried out by writing below the reading of the volume the same figure multiplied by two, but displaced to the right by two decimal places, and then subtracting to an accuracy of three decimal places. Methods of contracted multiplication should be used. The difference so obtained represents the true volume of nitrogen :—

2·280 mgm. of nitro-benzene yielded at 712 pressure and a temperature of 22° C. a nitrogen reading of		0·246 c.c.
2 per cent. of this	=	0·00492 ,,
True nitrogen volume . . .	=	0·24108 ,,
The same to three decimal places .	=	0·241 ,,

We always express the results of the nitrogen determinations as follows (using the figures of the above example) :—

2·280 mgm. (712 mm., 22° C.) yielded .		0·241 c.c. N.
Percentage nitrogen found . .	=	11·46 per cent.
Theoretical percentage . . .	=	11·41 ,,

Very occasionally substances are met with which form almost incombustible nitrogenous charcoal on heating with copper oxide in a current of carbon dioxide. After many experiments based on

the well-known macroanalytical methods, a process was finally found which enabled nitrogen to be correctly determined in such substances without any difficulty. It consists in mixing the substance, which has been weighed into the mixing tubes with an accuracy of three decimal places, with two or three knife points full of very finely-powdered potassium chlorate and then with ordinary finely-divided copper oxide in the usual amount. The charging of the tube and the subsequent combustion are carried out exactly as usual. The residual nitrogenous carbon burns completely in the evolved oxygen with liberation of the contained nitrogen which would otherwise escape measurement, and thus lead to a negative error. The circumstance that a large quantity of metallic copper is present in the hottest portion of the tube avoids any possibility of the introduction of oxygen into the azotometer, as the red-hot copper forms an impervious barrier to the oxygen, provided that the standard velocity of the gas current is not exceeded.

The volumetric determination of nitrogen in liquids can be carried out with the greatest accuracy by microanalysis, and perhaps with greater ease than by macroanalysis. The necessary quantity of liquid to be examined is weighed into a sealed capillary, together with a minute amount of potassium chlorate, in exactly the same manner as has already been described in detail for the determination of carbon and hydrogen. The combustion tube is charged in exactly the same manner as for other nitrogen determinations with the one difference that, in that section in which the mixture of the substance and finely-divided copper oxide is ordinarily placed, a cylindrical, freshly-oxidised roll of copper gauze, about 40 mm. long and of 5 mm. diameter, is introduced as a protection for the capillary. Immediately after breaking off the handle and point of the capillary, this is placed in the little roll and both are then introduced into the tube together. They are quickly followed, as usual, by a layer of coarse copper oxide. The analysis is carried out as usual and theoretical values are obtained.

V. The Determination of Nitrogen by Kjeldahl's Method in Small Amounts of Material. (Micro-Kjeldahl Method.)

In order to analyse by this method, solid substances are weighed from a weighing bottle in amounts of 3 to 5 mgm. just as for the micro-Dumas determination. They are then introduced into a

small decomposition flask and heated over a small flame after adding about 1 c.c. of concentrated sulphuric acid, a knife-point full of potassium sulphate and a similar amount of copper sulphate.[1] The decomposition usually takes place in an astonishingly short time.

It is well known that, to obtain correct results, it is often necessary to heat for a considerable time, and more particularly to ensure that elementary carbon is present in the sulphuric acid in order continually to produce water by decomposition of the latter. After the contents of the flask have become clear, two to three drops of alcohol are added from a wash-bottle for the purpose just indicated, and heating is then resumed. The amount of carbon introduced by means of this alcohol suffices to prolong the duration of the reaction by five to ten minutes. When the contents of the flask are once more perfectly clear, the ammonia which has been formed may be distilled without further delay.

If the organic material is decomposed in the actual distillation flask, transference and rinsing out of the reaction vessel are avoided, but for reasons of economy I prefer to carry out the decomposition in hard glass test tubes of the usual dimensions, the glass ends of which are blown out to a small bulb of 30 mm. diameter. After the reaction is completed, the contents of the tube are cooled, diluted with water, and rinsed into the bulb of the distillation apparatus. It is better to avoid using this latter for the high temperature reaction on account of the expensive ground glass joint. The distillation apparatus (Fig. 21) has undergone certain alterations compared with the one originally described by me ; the distilling flask, *K*, and its headpiece, *A*, have been increased in size and connected by an airtight ground-glass joint.[2]

One should never neglect to lubricate the ground joint with vaseline as otherwise it is liable to seize, owing to the action of the boiling caustic solution. The vertical condenser is now provided with a small cooling jacket. The use of a cooling jacket is particularly desirable as, when this is used, the condenser tube may be constructed of Jena resistance glass, instead of quartz, as under such conditions no appreciable amount of alkali is removed from the glass and the former necessity of steaming out the tube is avoided.

As will be seen from the drawing, this distilling apparatus is

[1] Ivor Bang made use of the same procedure, excepting that he added two to three drops of a 5 per cent. solution of copper sulphate.

[2] Through the instrumentality of Mr. Eugen Kolisko this apparatus is supplied by the firm of Paul Haack, of Vienna, IX, Garelligasse 1.

supported by two clamps, held by bosses screwed to a horizontal iron bar, which is adjustably affixed by a boss to the upright of a retort stand. One of these clamps holds the headpiece of the

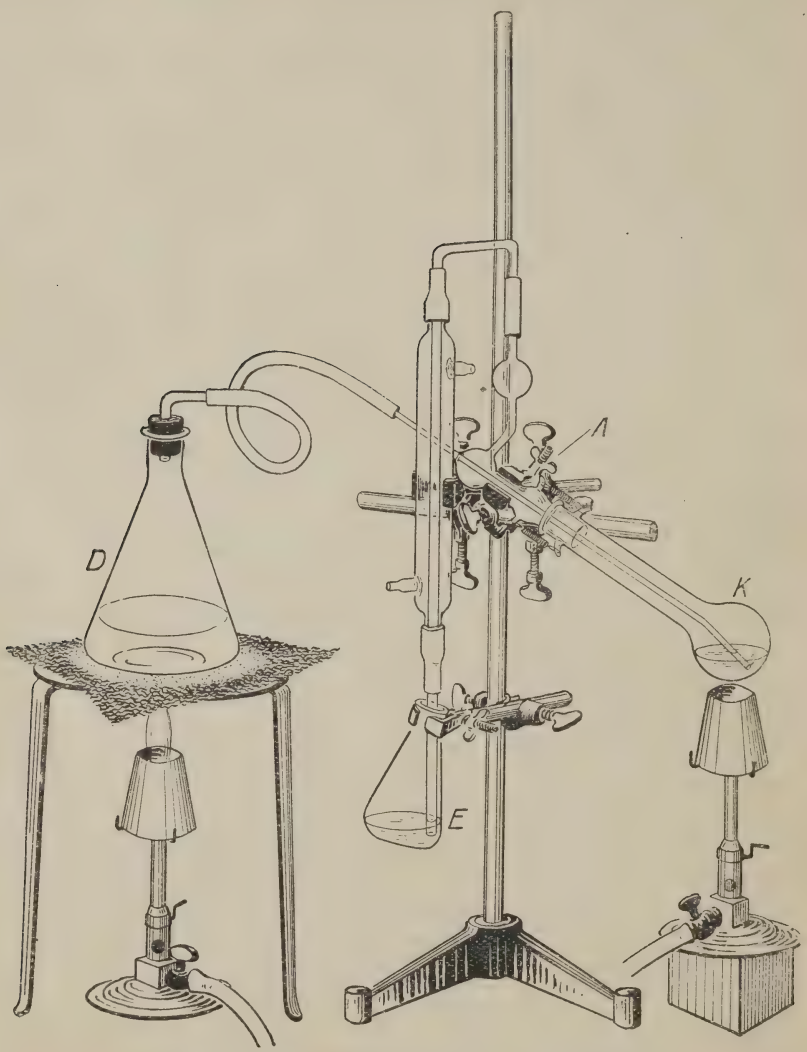

FIG. 21.—Micro-Kjeldahl apparatus. ($\frac{1}{5}$ actual size.)
D. Steam generator. *K.* Distilling flask. *A.* Distilling head. *E.* Conical flask with acid, used as receiver.

distilling flask in an oblique position, and the other holds the condenser. A third clamp, which is attached directly to the upright of the retort stand, holds the small receiving flask, *E*, containing acid in which the end of the condenser is immersed.

N/70 solutions are best used for the titrations, and as indicator we use methyl-red (*p*-dimethylaminoazobenzene-*o*-carboxylic acid) dissolved in a slightly insufficient quantity of N/10 NaOH solution. For preparing the standard solutions, N/10 hydrochloric acid and N/10 sodium hydroxide are used and adjusted with the help of the indicator. 28·6 c.c. of either solution are run into a 200-c.c. measuring flask from a burette ; one to two drops of the indicator solution are added, and the measuring flask is then filled with water up to the mark. When so prepared, the N/70 caustic soda is canary-yellow in colour, whilst the N/70 hydrochloric acid is rose-red.

Pinchcock burettes of 10 c.c. capacity, and divided to ·05 c.c., have been found very suitable for the titration. They are furnished with jets consisting of narrow glass tubes, which are drawn out to capillaries of narrow bore about 5 to 8 cm. long, and of an outer diameter of about 1 mm. The fluid friction in these jets is so high that the drops are only discharged very slowly, even when the pinchcock is open, and it is therefore easy to discharge quantities of 0·01 c.c. of liquid from such burettes, and to estimate such amounts by the position of the meniscus, particularly if a lens is used.

One cubic centimetre of a N/70 solution corresponds to 0·2 mgm. of nitrogen ; therefore the smallest amount of nitrogen which can be determined by titration, corresponding to 0·01 c.c., is 0·002 mgm. of nitrogen.

If the apparatus has not been used for some time, it is advisable to clean it thoroughly and steam it out with the help of the steam generator. The small conical flask used as a receiver should also be steamed out before use, although of Jena glass. This is charged with a more than sufficient quantity of N/70 hydrochloric acid (from 3 to 6 c.c.), and is clamped to the stand so that the acid just covers the end of the condenser tube.

Thirty per cent. sodium hydroxide solution is added to the contents of the distilling flask, through a small funnel attached to the end of the rubber tubing which ordinarily connects it with the steam generator, in such quantity that a little copper hydroxide is precipitated. Distillation is then started by connecting this tubing with the previously heated steam generator. In order to promote uniform boiling of the generator, which consists of a large conical flask, a few small spoonfuls of zinc dust are previously added to the water. Neither steam distillation alone, nor a current of air, suffice to drive the whole of the ammonia over into the receiver

quantitatively, and it is necessary that the liquid to be distilled should be maintained at the boil. This is achieved by means of a small flame under the distillation flask. After ten to fifteen minutes the receiver containing the distillate is lowered to such a point that the mouth of the condenser is about 1 cm. above the level of the liquid. The condenser tube is completely rinsed out by the further distillate in less than five minutes. The distillation is ended by removing the tubing from the steam generator. Both burners are removed, the outside of the end of the condenser is rinsed with the wash-bottle, and a further small quantity of undiluted indicator solution is added to the liquid by means of a glass thread. Since the introduction of the use of a cooling jacket to the condenser, it has been found that the carbon dioxide of the air influences the result unfavourably in spite of the insensitiveness of the indicator solution, and it is therefore necessary to boil the acid distillate thoroughly again before titrating.

Fig. 22.—Micro-pipette. (Actual size.)

The excess of acid in the liquid must be titrated until a permanent canary-yellow coloration is obtained. As this tint reverts slightly during the first minute, one has time definitely to ascertain that the end point has been reached by comparison of the colour of the flask contents with that of the N/70 sodium hydroxide solution in the burette. In this manner, the titration can be carried out with ease and exactitude, either by daylight or artificial light.

The weight of nitrogen in milligrammes is obtained by multiplying the number of cubic centimetres of N/70 hydrochloric acid neutralised in the distillate, which has been determined to two decimal places, by the factor 0·2.

In my first communication I already referred to a precision pipette for use in the determination of nitrogen in liquids, such as urine. The one illustrated in the accompanying figure (Fig. 22), is superior in form and convenience to that formerly described. Its widened portion, between the ring-marking and the finely drawn-out point, has a capacity of 0·15 c.c., which should be controlled by careful weight calibration with mercury, using the necessary temperature correction. A portion of the liquid measured with this pipette necessarily adheres to its inner wall. It is

necessary, therefore, to rinse out the bulk of this residue with a few drops of water introduced from above ; as a further precaution, the concentrated sulphuric acid used for the decomposition is also passed through the pipette in order to remove the last traces of liquid completely.

I. K. Parnas and R. Wagner[1] have adapted the above described distillation apparatus in very convenient fashion for rapid consecutive determinations, as they convinced themselves that it is the most efficient apparatus hitherto described, and that the alkali is retained even when the distillation is very rapid. M. Richter-Quittner has also expressed a favourable opinion on my method of decomposition and distillation. I will now quote

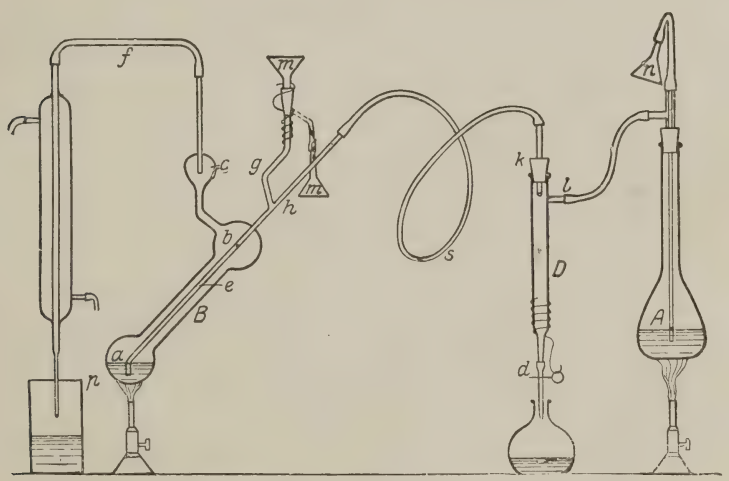

FIG. 23.—Distillation apparatus of Parnas and Wagner.

verbally Parnas and Wagner's description of their method (Fig. 23) :—

" The distilling flask, *B*, is expanded at its upper end to a bulb, *b*, which is about equal in size to the lower bulb, and into which is sealed a delivery tube, *e*, for the steam. The outlet tube is sealed on to the upper bulb, as in the extension of Pregl's flask, and is furnished with a Hopkins distillation head, *c*. The steam inlet tube, *e*, ends exactly at that point in the flask which corresponds to the deepest point in the liquid, so that the latter can be completely removed by suction. This tube is forked above the flask. The upper limb, *g*, is connected by rubber tubing with a small funnel, *m* ; the lower limb, *h*, which rises to a height not less than

[1] " On the Determination of Small Quantities of Nitrogen by Kjeldahl's Method," *Biochem. Zeitschr.* (1921), **125**, 253–256.

the top of the limb, *g*, is connected by means of a fairly long rubber tube, *s*, with a glass tube, *k*, which is inserted in a cork stopper. This cork stopper closes a cylindrical receiver, *D*, the lower end of which is closed with rubber tubing and a pinchcock, and which is also provided above with a side tube, *b*, through which it is connected by means of rubber tubing with the steam generating flask, *A*. This steam generating flask is furnished above with a T-tube, the horizontal limb of which leads to the rest of the apparatus, whilst the vertical limb is connected by soft rubber tubing with a funnel, *n*. Lengths of soft rubber tubing, into the top of which a small funnel is inserted, form convenient valves ; when bent downwards by the weight of the funnel they close the tubes tightly.

"The steam generator, *A*, is heated and the escaping steam is allowed to escape through the open jet, *d*. Meanwhile the distilling flask is filled with the decomposition products which have previously been diluted with water. As a precaution, the rubber tubing, *s*, is closed by pinching it. The funnel, *m*, is raised, and the liquid poured into it and rinsed down with a little water. The end of the condenser is then immersed in the contents of the charged receiver, *p*. The caustic potash solution, to which a little thiosulphate has been added, is also poured in through *m*, and rinsed down with a little water, after which the funnel is allowed to drop, and *a* is heated with the micro-burner. Steam is now allowed to pass through the tubing, *s*, whilst *d* is closed, and the ammonia is distilled in a vigorous current of steam. As soon as the distillation is finished, the receiver and the burners under *A* and *a* are removed. The contents of the flask, *a*, are consequently sucked back into *D*, and can be withdrawn by opening *d*. Water can then be added through *m*, in order to rinse *a*, after which it is similarly sucked back into *D* and withdrawn. The apparatus is then ready for the next charge. As each determination only requires a few minutes a series of determinations can be carried out without disconnecting the apparatus.

"In our determinations we used N/100–H_2SO_4 with 0·04 N potassium iodate in the receiver and titrated back by Kjeldahl's method. We added a trace of methyl-red to the sulphuric acid before distilling."

I tested the apparatus of Parnas and Wagner, which has just been described, and this gave me an opportunity to revise all the conditions for the micro-Kjeldahl determination. Whilst the whole of the rest of the apparatus was found to be very satis-

factory, the hard glass condenser tube which was provided caused an error due to the glass, which was not completely eliminated after several days' steaming, and I was therefore obliged to replace it by a quartz tube of about 7 mm. outer diameter. This was bent twice in such a manner that the shorter vertical limb, 5 cm. long, was connected with the long descending vertical limb, which was about 35 cm. long, by an intermediate portion 15 cm. long, which was inclined upwards towards the latter. The longer limb was surrounded in its upper portion for a length of about 20 cm. by a glass cooling jacket of $2\frac{1}{2}$ cm. diameter. The error which was due to the glass was completely eliminated by the use of the quartz tube. It should be specially noted that Pilch,[1] who was the first to carry out microanalytical nitrogen determinations in organic substances by Kjeldahl's method, had in view the elimination of errors due to glass, and used a metal condenser with a platinum tube. Somewhat later, Ivor Bang recommended a silver condenser tube in place of one of platinum. Now, however, the price of both metals renders their use almost impossible, and it is satisfactory to be able to replace them perfectly by quartz, which offers the great convenience that one is enabled to use a longer tube, and therefore to work with flasks of 25 to 50 c.c. capacity in spite of their height.

In conjunction with Dr. Hans Wostall, I compared the acidimetric titration, with methyl-red as indicator, which has just been described, with the iodimetric method used by Ivor Bang.[2] As is well known, the latter method is carried out by distilling into N/100 acid solution, after which 2 c.c. of a 5 per cent. potassium iodide solution and two drops of a 4 per cent. potassium iodate solution are added to the contents of the receiver. According to Bang, it is then necessary to wait for five minutes, so that the separation of iodine may be completed; the solution is then titrated back with N/200 thiosulphate solution. We preferred to add the starch solution just before the end point. These experiments showed us that the acidimetric titration with methyl-red as indicator is scarcely inferior to the iodimetric method, if freshly-steamed hard glass flasks are always used, the solution boiled before the end point is reached, and if care is taken that the final canary-yellow tint remains unaltered for some minutes; for 0·01 c.c. of a 0·01 N solution appreciably affects the tint. On the other hand the acidimetric titration offers the advantage, com-

[1] *Monatsh f. Chemie* (1911), **32**, 26.

[2] "Methods of Micro-determination of various Blood Constituents." (Wiesbaden, 1916.) (J. F. Bergmann.)

pared with the iodimetric method, that it is possible to titrate back at any time if the end-point has been passed, and thus to obtain the correct result.

We also convinced ourselves that, when using the apparatus of Parnas and Wagner, quantities of nitrogen amounting to several milligrammes can be quantitatively transferred to the distillate in 2 minutes. In practice, therefore, we distil for 3 minutes with the end of the condenser below the surface of the liquid in the receiver, and then continue to distil for a further minute after the latter has been lowered.

In general it is found that the use of copper sulphate as an oxidising catalyst when decomposing the organic matter is completely effective. In exceptional cases, more particularly for certain albumenoids, mercury is necessary, preferably in the form of mercuric sulphate, in order to transform the whole of the nitrogen into ammonia. In such cases sodium hydroxide scarcely suffices to liberate the ammonia from the mercuri-ammonium compounds, and sodium sulphide was formerly used for the purpose. The use of this reagent is quite inadmissible, particularly if the iodimetric titration is practised, as hydrogen sulphide passes into the distillate. Parnas's very practical method of adding thiosulphate to the sodium hydroxide should therefore be adopted. This reagent separates the metals as sulphides both from mercury and copper salts, and thus appreciably reduces the period necessary for the distillation without risk of not completing the operation.

VI. Microanalytical Determination of the Halogens and of Sulphur

In spite of the fact that the determination of these elements in a few milligrammes of organic material by the method of Carius had always yielded satisfactory results up to that time, the late Dr. Emil Schwinger (whose premature death is greatly to be deplored) and I, when working together at my Innsbruck laboratory in the autumn of 1912, had to deal with a substance with which correct halogen values could only be obtained after heating for several days to 300° C. with concentrated nitric acid. This substance was a chlormethylanthrone which had been sent to me by Professor Scholl. This circumstance induced us to develop a method in which the organic substance was rapidly and completely destroyed by combustion in a current of oxygen, and the halogen isolated as silver halide in the products of combustion and weighed. After a few preliminary experiments, Schwinger and I obtained practical

results by passing the products of combustion over red hot platinum foil, and then over porcelain beads which were moistened with sodium carbonate solution containing sodium bisulphite. The sodium bisulphite served the purpose of reducing any sodium halogenate or hypohalogenite to sodium halide. A precipitate of silver halide was obtained from the washings of the porcelain beads by adding a mixture of silver nitrate and concentrated nitric acid ; the latter ensured the oxidation of the sulphite to sulphate. At that time we already used the older form of filter tube and automatically transferred the silver halide precipitate on to the asbestos filtering layer.

Sulphur determinations were also carried out on the same principle ; in this case, also, the products of combustion of the organic material were passed over red hot platinum and then over porcelain beads which were moistened with sodium carbonate solution, to which a few drops of perhydrol (hydrogen peroxide) had been added. The sulphur could then readily be determined in the washings of the porcelain beads by acidifying with hydrochloric acid and precipitating as barium sulphate by adding barium chloride to the hot liquid. The principle of this method of determining sulphur has proved completely satisfactory up to the present, and apart from a few details, it is now carried out in the same manner as the first successful experiments in 1912.

The development of the new method of determining halogens followed a very different course, as it was only discovered after numerous and arduous experiments that even if all the reagents were free from halogen the blank tests still produced a weighable precipitate. The explanation of this fact is really comparatively simple ; it is due to the circumstance that on acidifying an alkaline sulphite solution with nitric acid, adding silver nitrate and heating for some time on the waterbath, an opalescence is produced which is not silver halide, but finely-divided sulphur. Evidently the oxidation of sulphurous to sulphuric acid is not a monomolecular but probably a multimolecular reaction, which probably gives rise to the production of polythionic acids as intermediate products which separate sulphur at a later stage. The discovery of this fact led to methods of avoiding the undesirable separation of sulphur. If sulphides are oxidised with perhydrol in alkaline solution, the reaction proceeds smoothly, and on adding nitric acid and silver nitrate, the solution remains perfectly clear, even if it is warmed for a long time.

During the tedious investigations of this essential source of

error in the original method of determining halogens, various other important observations were made which led to the elimination of a number of other small sources of error, which were, however, sometimes additive in their effect. During this work, all the major and minor conditions were completely investigated and methods were discovered of eliminating possibilities of error. We thus finally developed a satisfactory process which is fully under control and the universal applicability of which has been proved by determinations made by Dr. Hans Lieb on the most various classes of substances.

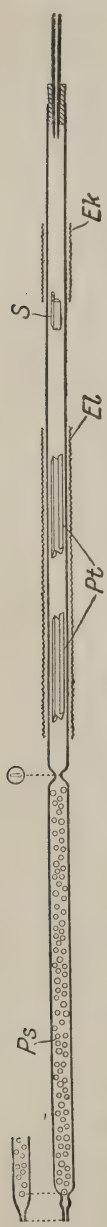

Fig. 24.—The combustion tube with bead filling as used for the determination of halogens and sulphur. (⅓ Actual size.) *Ps.* Porcelain beads. *Pt.* Platinum contacts. *El.* Long roll of iron gauze. *Ek.* Short roll of iron gauze. *S.* Boat.

In comparison with methods of determining halogens which have previously been used, the method now under discussion offers a number of advantages which are worth mentioning :—

1. The time required for the complete decomposition of the material and for the whole determination is much shorter than for any other method.

2. The possibility of simultaneously determining residues in salts by re-weighing the boat and contents. This is particularly applicable to the salts of gold and platinum.

3. The possibility of determining halogen without difficulty in substances which are decomposed by cold nitric acid or its vapours.

The Combustion Tube with Bead-filling.
(Fig. 24.)

The decomposition of the weighed organic substance is carried out in a combustion tube of Jena resistance glass, which is at least 500 mm. long. The tube is drawn out at one end to a thick-walled point, the bore of which should not be more than 0·5 mm. The object of this fine opening is to prevent the washings, which are produced after the completion of the combustion, from running through the tube too quickly, so that the necessary time of contact with the beads may be ensured, thus rendering it possible to rinse the beads quantitatively with small amounts of liquid. In order to prevent the point from splintering,

it is advisable first to grind it with coarse emery cloth, and finally to polish it with fine rouge paper. In the region of the conical portion of the drawn-out point, there are two parallel depressions in the tube, which prevent a porcelain bead from lodging at this point and obstructing the washing process. The adjacent portion of the tube is filled to a length of 200 mm. with porcelain beads, *Ps*, of about 3·5 mm. diameter. The porcelain beads must not be porous, as otherwise it would be almost impossible to wash them thoroughly.

In order to prevent the beads from falling out, the tube is provided with two more parallel depressions behind these which reduce the cross-section just sufficiently for the purpose. If the cross-section at this point is too narrow, it becomes difficult to fill that portion of the tube containing the beads with water without an air space.

It is advisable to allow a new tube to stand for some days filled with distilled water, in order to remove any soluble silicates which may be present in the beads.

Before each determination, the tube is cleaned by filling it with sulphuric-chromic acid by suction with the mouth from the narrow end, and allowing the acid to run out at the point after revolving the tube. The tube is then washed successively within and without with tap water, distilled water and alcohol, and is finally dried on the air pump by connecting the latter with the point by means of rubber tubing, attaching an air filter to the wide opening of the empty portion and carefully warming the whole with the flame.

The Two Platinum Contacts

It is best to make these in the laboratory from pieces of platinum foil, 0·05 mm. thick, 50 mm. long, and 15 to 18 mm. wide. These are so bent longitudinally that the cross section forms a large Latin " Z " with somewhat lengthened initial and end strokes. For this purpose two parallel lines, 5 mm. apart, are drawn on a large sheet of paper, and the platinum foil placed longitudinally over this, so that its centre is half-way between the two lines and that it extends equally on either side. Points are also marked which bisect the distances between the parallel lines and the edges of the platinum strip. At these points and lines the foil is bent longitudinally by pressing on to it a piece of pasteboard with a straight edge, and bending the projecting portion over. When the bends have once been commenced, they are easily completed

by hand until the whole contact, which is 50 mm. long, has throughout the above-mentioned cross-section of a Latin "Z." In spite of the small thickness of the material it offers considerable resistance to distortion and bending. These contacts are easily pushed into the combustion tube. They may easily be removed with the help of a hook of platinum wire, sealed into a long glass rod, particularly if the ends of the contact are slightly bent over, or if the edges at the narrow end of the foil are provided with perforations at one or two places, into which a hook can be inserted.

Both platinum contacts must be boiled in dilute nitric acid, and fully ignited in a flame before use, and are then at once placed in their correct positions in the combustion tube.

It sometimes happens that these contacts are "poisoned," that is, that they lose their catalytic activity; this may be restored by etching them with hot aqua-regia.

The contacts should always be kept in a glass dish covered with a clock-glass, and should not be handled except with platinum tipped forceps.

It is to be hoped that the firm of Heräus, of Hanau, may supply 4 to 6-rayed platinum contacts, 50 mm. long, to fit the tubes which are used.

The Filter Tubes

Until 1912, I used a micro-Gooch crucible for filtering the precipitates of silver halide; this was soon replaced by the Neubauer crucible. A wish to convey the silver halide precipitates automatically on to the filtering surface led to the construction of a filter tube suitable for the purpose. Its construction will be clear from Fig. 25A, which also shows the platinum wire bent to a flat spiral, which was placed at the bottom of the upper portion of the filtering tube and served as a support for the layer of asbestos which was consolidated above it by suction. This small apparatus still had certain defects; more particularly the layer of asbestos in the cylindrical portion swelled and underwent distortion when moistened, and this sometimes caused leakage between the filtering layer and the glass wall. This defect was completely avoided by the new form of filtering tube, Fig. 25B, which has been in constant use with entirely satisfactory results since the spring of 1914.

Its widened upper portion is prepared from a hydrometer tube 9 mm. wide, into the end of which is sealed a stout glass tube 4 mm. wide. The bore of the tube is constricted to 0·5 mm. at the junc-

tion of these two portions, whilst above this constriction the wider portion is blown out to a diameter of about 11 mm. to a height of about 4 mm., thus forming a shallow cavity at the junction of the narrow tube which serves to hold the asbestos pad, the edges of which are prevented from rising at the circumference when it is moistened. Above this space, which holds the filtering material,

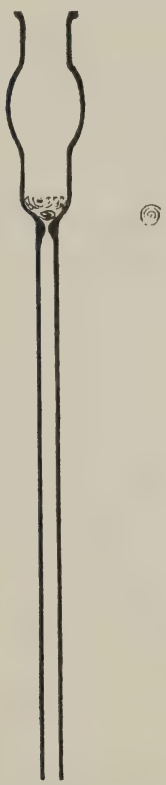

FIG. 25A.—Earlier filtering tube, with spiral of platinum wire.

FIG. 25B.—New form of filtering tube. (Actual size.)

there is a second expanded portion 15 mm. long, and also of a diameter of 11 mm., above which there is a short neck with a rim. The length of the wide portion of the filtering tube is about 35 mm. It is convenient to give the narrow tube a length of 80 to 90 mm. and to cut it down, after the tube has been charged with the asbestos pad, to such an extent that the tube can be suspended on the hooks on the left pan of the microanalytical balance, with-

out appreciably dragging the pan to one side on releasing the arrestment.

Robert Strebinger recently recommended the construction of such filtering tubes from quartz glass, in order to enable the filtered precipitates to be ignited. With regard to this difficult process, I must refer to his publication in the *Österr. Chemiker-Zeitung* **21**, 71, (1918), and also to Emich in *Abderhalden's Handbuch der biolog. Arbeitsmethoden*, 1921, div. I., Volume 3, Part 1, p. 279.

The filtering tube is prepared for use by introducing commercial Gooch crucible asbestos into the tube in a dry condition, and pressing it down with a sharp-edged glass rod, in such a manner that the receptacle intended for this filtered material is completely filled, particularly at the circumference. The tube is then twice filled on the filter pump with a thin suspension of asbestos in water, and thoroughly washed with water in order to completely remove any adhering particles of asbestos ; care is then taken that the quantity of asbestos is sufficient to completely fill the space intended for the purpose. The asbestos pad is then washed in the following manner :—First with hot sulphuric-chromic acid, then with water, then with hot nitric acid and water, and finally with alcohol. After this treatment, the tube is dried by aspirating an air-current through it. This operation is carried out in an apparatus which was originally used for drying the older form of absorption tube, and was therefore called the regenerating block (Fig. 15). As will be seen from the drawing, it consists essentially of two superposed copper blocks with a polished interface, each of which is provided with semi-circular channels, which together form tubular perforations when the blocks are superposed. One of these perforations has a diameter of 12 mm., and serves to hold the widened portion of the filter tube, and the other has a diameter of 8 mm. These two copper blocks are heated from below with a very finely adjustable micro-burner. The temperature can be read on a thermometer, which fits horizontally into a socket intended for the purpose in the lower copper block. The micro-burner is provided with a delicate regulation, by means of which the temperature can be maintained constant for days to within 2° to 3° C. In order to dry the tube, the outer surface is wiped with a clean cloth, the stem is inserted into a small piece of rubber tubing which is connected by a glass connecting-piece with the rubber tubing reaching to the water pump. The inside of the tube is, of course, still moistened with alcohol. The mouth of the filter tube is then provided with an air-filter in order to avoid increase of weight

caused by the dust particles which are always present in the air of the laboratory. This air-filter consists of a cork which fits into the neck of the filter tube, and has a central perforation through which passes the short end piece of a tube, 30 mm. long and 8 to 9 mm. wide, the interior of which is filled with compressed and defatted cotton wool. Silver halide precipitates are best dried at a temperature of 120° to 125° C. The precipitates and asbestos are dried in not more than five minutes by aspirating through them a slow current of air whilst the tube is placed in the wide channel of the regenerating block. It is then advisable to place the stem in the narrow channel, for a few minutes, in order to avoid the retention of condensed liquid in that portion. After drying in the above manner, the filter tube is disconnected from the pump and from the air filter, and is then wiped with moist flannel, then with chamois leather as was described in the case of the absorption tubes, after which it is placed directly in the balance case.

In consequence of the large amount of asbestos this apparatus requires thirty minutes in order to attain constant weight. If it is protected from dust, the weight last noted may be taken as the initial weight.

We used these tubes for filtering and weighing all three silver halides consecutively, as all three require the same treatment. When the total amount of silver halide precipitates has reached 50 to 60 mgm. filtration becomes difficult, and it is then advisable to remove the silver halides with concentrated potassium cyanide solution. This is then displaced by water, and the filter tube washed as already described with warm sulphuric-chromic acid, water and alcohol, each liquid being poured in twice in succession into the upper portion of the filter tube to the upper rim.

Reagents

Obviously all reagents used in the halogen determinations must be absolutely free from halogen. The solutions which are used must also be perfectly clear and free from suspended particles. It is therefore advisable not to add the necessary solutions directly from the stock bottles, but first to pour them into test tubes and to use them if they are then found to be of satisfactory quality.

Distilled Water

The preparation of this necessitates a little care. 10 c.c. when mixed with 5 drops of nitric acid and the same quantity of silver nitrate solution, and warmed for ten minutes on the boiling

water-bath, should show no trace of opalescence. It is advisable to close the mouth of the stock bottle with a soda-lime tube in order to prevent access of laboratory air.

Concentrated Nitric Acid

This often contains chloride. It is therefore best to prepare an acid free from chlorine by distillation over silver nitrate. This is best carried out in a tubulated retort, through the tubulus of which passes a glass tube drawn out to a capillary reaching to the bottom of the retort, and through which is passed a current of carbon dioxide from a Kipp generator, the gas being first washed with sodium carbonate solution. The nitric acid free from chlorine, which is thus obtained, is best kept in a brown bottle with a stopper and glass cap to avoid contamination by the air of the laboratory.

Sodium Carbonate Solution Free from Halogen

We had much trouble in preparing sodium carbonate solution free from halogen until our attention was called to the excellent method of B. Reinitzer (*Zts. f. analyt. Chem.*, **34**, 575, 1895).

500 gm. of commercial sodium bicarbonate are stirred up with a little water to a paste ; this is drained on a Buchner funnel, and the operation repeated until the last filtrates show scarcely any chloride reaction. The sodium bicarbonate, which has been washed in this manner, is added with stirring to about 2000 gm. of water at 80° C. in a Jena glass beaker until a portion remains undissolved. After each addition there is a strong evolution of carbon dioxide. The hot solution is then filtered through a folding filter, which has previously been carefully washed with distilled water free from halogen, and the filtrate is cooled as much as possible. After standing for some time, a salt crystallises out, of the composition $Na_2CO_3 + NaHCO_3 + H_2O$. This is drained on a Buchner funnel, washed with a little water, and tested by suspending 1 gm. in water, adding nitric acid free from halogen and silver nitrate, and then heating for five minutes on the boiling water-bath. If the operation has been carried out successfully, not the slightest opalescence should be visible even with oblique illumination. Should such an opalescence be present, the product must once more be recrystallised from water at 80° C. The perfectly pure salt is added to boiling distilled water in such quantity that a saturated solution is obtained ; this is poured into the stock bottle while still warm. A further quantity of pure

sodium carbonate crystallises in the bottle. When the solution has been used up, a further quantity of suitable solution can be obtained by adding pure distilled water to the crystalline sediment. I always protect the glass stopper of the stock bottle with a solution of paraffin in benzene to prevent it from seizing, and place an inverted beaker over the neck of the bottle in order to protect its mouth from dust.

Bisulphite Solution Free from Halogen

This solution is prepared from the concentrated sodium carbonate solution free from halogen by slowly introducing sulphur dioxide free from halogen, avoiding any heating, and preferably with cooling. Should the temperature rise during the introduction of the sulphur dioxide, appreciable quantities of thiosulphate are often formed, which give rise to the separation of sulphur on acidifying. The sulphur dioxide is prepared in a small generator by slowly adding sulphuric acid to commercial bisulphite, and passing the liberated sulphur dioxide through a tube containing glass wool, which has been moistened with concentrated sodium carbonate solution free from halogen, before it is introduced into the cooled sodium carbonate solution.

The sodium bisulphite solution, which is so obtained, is stored in test tubes which are half filled and then sealed in such a manner that a long capillary is produced. A considerable quantity of pure sodium bisulphite solution can be stored in this form. It might be possible for one of our large chemical firms to supply this preparation. Before use, the drawn-out point of the capillary is opened, and the solution is expelled, drop by drop, by warming with the hand. The well-wiped point may then be closed by a drop of paraffin, or by sealing in the flame.

In order to comply with the necessary requirements for the determination of halogen, this solution should conform to the following test: 20 to 40 c.c. are made alkaline with sodium carbonate solution free from halogen and warmed on a water-bath with 3 to 5 drops of perhydrol for five minutes. After cooling a mixture of 1 to 2 c.c. of nitric acid free from halogen and 0·5 c.c. of silver nitrate solution is added and the liquid is heated for ten minutes on the boiling water bath. No trace of turbidity should be produced.

It is very desirable that one of our large firms of suppliers of laboratory requisites should supply solutions of sodium carbonate and of sodium bisulphite free from halogen in sealed glass tubes,

which might contain about 20 c.c. each, as the preparation of these solutions in the laboratory takes a considerable time.

Silver Nitrate Solution

A 5 per cent. solution of this substance is prepared from commercial crystallised silver nitrate. Any turbidity settles overnight and does not affect its suitability.

The Course of the Halogen Determination

A wide test tube (Fig. 26, *R*), of about 25 mm. diameter, is carefully cleaned with sulphuric-chromic acid and water, and is then charged with a mixture of 2 c.c. of concentrated sodium carbonate solution free from halogen, and 3 drops of the pure bisulphite solution. This solution is aspirated into that portion of the combustion tube containing the beads, which is inclined in such a manner that the liquid moistens all the beads. The excess of liquid is then expelled, and the portion of the tube filled with the beads is protected from contamination by covering it with the wide test tube, both the combustion tube and test tube being held in the right hand. After closing the wide mouth of the combustion tube with a plug of cotton-wool in order to protect it from particles of iron, two rolls of iron gauze are slipped over it with the left hand, the first being 150 mm. and the other 35 mm. long. These should not fit too tightly. The shorter roll is placed over that portion, 14 cm. long, which is subsequently to be heated with the tube burner. The combustion tube is then placed on the combustion stand, and the wide-mouthed end which projects beyond the stand may conveniently be held in the clamp of an ordinary retort stand. At the other end of the combustion stand about 5 cm. of the empty portion of the tube behind the constriction should project, apart from the portion filled with the beads. This projecting portion, part of which lies in the protecting test tube, is supported by suitable forked stands. This is very necessary in order to prevent serious distortion during heating. The cotton-wool plug is then removed, and the two freshly ignited platinum contacts are introduced, one behind the other, into the empty portion of the tube which is surrounded by the roll of iron gauze, which reaches to the front end of the combustion stand. The substance to be analysed, which has already been weighed into a boat, is now introduced as already described in connection with the carbon and

hydrogen determination, and is pushed so far forward that there is an interval of at least 5 cm. between the boat and the long roll of wire gauze. The open end of the tube is closed with a well-fitting perforated rubber stopper, into the perforation of which is fitted a tapered glass capillary for the introduction of oxygen. The oxygen is supplied from an ordinary gas-holder, and passes through a wash-bottle containing a suspension of sodium bicarbonate before entering the tube. For this determination the rubber tubing does not require any preliminary ageing treatment as for the carbon determination. It is, however, convenient to introduce into the rubber tubing connecting the wash-bottle and the combustion tube a little twisted cotton-wool or a few lengths of string, in order to facilitate regulation by a screw pinchcock, so that a bubble frequency of two bubbles per second may be maintained.

The platinum contacts are now heated by lighting the tube burner, keeping the flames low at first. This portion of the tube is covered with the usual coarse wire gauze to reduce radiation. When the contacts are red hot one may commence to heat the material in the boat. For this purpose the front portion of the small roll of wire gauze is approached to within 5 or 10 mm. of the boat, according to the volatility of the material, and the middle of the gauze is then heated with the movable burner which is barely non-luminous. The analyst will then be able to observe the changes in the burning material. It is necessary carefully to avoid advancing the flame or the hot gauze until any changes due to the previous position of the burner are completed. Substances, such as tribromophenol, which distil on heating, require particular care, as distillation commences as soon as they are melted and the distillate collects in the form of a perfectly clear drop in the empty space between the boat and the red-hot portion of the tube containing the platinum contacts. After a short time the boat is empty, and it would be quite wrong to heat it along its whole length, as if this were done the drop of condensed material already referred to would be vaporised far too rapidly, very probably leading to an explosion and consequent projection of unburnt material beyond the red-hot platinum contacts, thus rendering the analysis useless.

When the whole of the material has been burnt in the manner described, and the movable burner has been moved up to the tube burner, all the burners are extinguished, and the tube allowed to cool in a current of oxygen. After removing the rubber

stopper the boat and the platinum contacts are removed from the tube. If the substance being analysed is a salt, more particularly of platinum or gold, the usual precautions must be taken in reweighing the boat. The open mouth of the tube is then closed with a cotton-wool plug. The clamp holding the tube is opened ; the protecting test tube and the bead-filled portion of the tube are held in the right hand whilst, with the other hand, the two rolls of wire gauze are removed, still maintaining the tube in a horizontal position, and it is wiped free from any adhering particles of iron with a cloth. The tube is then clamped into a stand at an acute angle with the vertical, in such a manner that the bottom of the test tube rests on the bench top and the point of the combustion tube takes up a position in the test tube about 4 cm. high. The plug of cotton-wool is now removed, 2 to 3 further drops of bisulphite solution are introduced into the tube, and water is added in a steady stream from a wash-bottle until it fills that portion of the tube containing the beads. This operation is sometimes attended with difficulties, if the expulsion of air is hindered by the constricted portion being too narrow. In such cases the difficulty may often be overcome by turning the tube about its axis. After running off the first washings the process should be repeated, and although the halogen contents are presumably removed by this operation in consequence of the long contact with the tube contents and beads, a third washing may follow in order to be certain. With careful work a total volume of about 30 c.c. of liquid only is thus obtained. After withdrawing the combustion tube its outside point should also be rinsed.

When determining iodine, elementary iodine often separates in that portion of the tube which projects beyond the combustion tube. In order easily to bring this into solution it is advisable to drive it into the bead-filled portion by careful heating and, during washing, to suck back the first washings into the obliquely held tube so that any elementary iodine may be dissolved. Before washing the tube for a second time with water 2 further drops of bisulphite solution should be introduced ; as will be mentioned later, a larger quantity of perhydrol than usual is then required.

The united washings in the test tube (Fig. 26, *R*), if to be used for the determination of chlorine or bromine, are treated with 2 drops of pure perhydrol in order to oxidise the sulphite, and are then warmed in the boiling water bath for three to five minutes

after inverting a beaker over the mouth of the test tube to protect the contents from dispersed drops of water. When iodine is being determined the liquid must not be warmed or iodate will be formed, but in this case a somewhat larger quantity of perhydrol is necessary for the oxidation of the sulphite (some 4 to 5 drops of pure perhydrol), after adding which the liquid is allowed to stand for ten minutes at the ordinary temperature. It should be mentioned here that the determination of iodine is one of the most difficult operations in microanalysis, as the slightest impurities, such as dust, increase the weight of the silver halide precipitates, and thus lead to considerably higher iodine values. In this connection it should be noted that the silver halide precipitates contain more than 50 per cent. of iodine.

These considerations apply equally to the determination of iodine by the method of Carius, and for this reason it is better to use rather larger quantities of material for iodine determinations, namely, 5 to 10 mgm.

After thoroughly cooling the liquid under the tap, a clear mixture, which has previously been prepared in a test tube, of 1 c.c. of concentrated nitric acid and double the quantity of silver nitrate solution, is added ; this produces a precipitate of silver halide which usually forms a fine turbidity in the first instance. On reheating for 10 to 15 minutes in the boiling water bath the silver halide balls together, and is then easily filtered after cooling completely. The liquid is best cooled under the tap and finally by immersing the covered test tube in a large quantity of cold water. It is to be observed that equally correct values are found, whether the precipitate is filtered ten minutes after complete cooling or after an interval of several hours.

Before filtration the properly washed and dried filtering tube is wiped with moist flannel and with chamois leather, left in the balance case or even suspended over the pan for at least twenty minutes to cool, and then weighed. It is weighed with an accuracy of 0·005 mgm. only ; it is impossible to determine the weight to within 0·001 mgm. on account of the influence of the atmospheric moisture on the large glass surface and the asbestos filter, and is no more necessary than in the case of the absorption tubes for the carbon and hydrogen determination.

The stem of the weighed tube is then passed through a previously moistened piece of thick-walled rubber tubing, 1 cm. long (Fig. 26, *S*), which fits a glass tube (Fig. 26, *G*) 70 mm. long, which fits in turn into the perforated rubber stopper of a

filtering flask in such a manner that its height can be adjusted as shown in Fig. 26. The precipitate is transferred to the filter by means of a syphon tube, which consists, as shown in the drawing, of a short limb and a long limb, 25 cm. long, parallel with the former, these being joined by a shorter length which falls towards the shorter limb. This syphon is made of glass tubing not more than 4 mm. in diameter. If the bore is too wide the liquid traverses the tube at a lower speed, and the silver halide precipitates then have time to drop back in the tube before

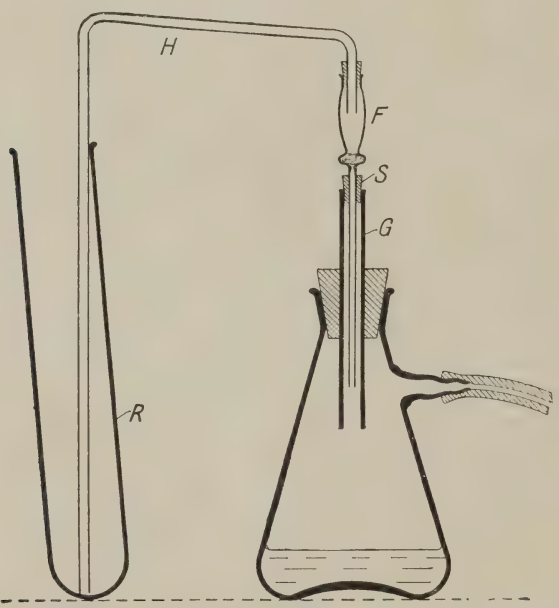

FIG. 26.—The transference of silver halide precipitates. ($\frac{1}{2}$ actual size.) *F.* Filter tube. *S.* Rubber tubing. *G.* Adjustable glass tube. *H.* Syphon. *R.* Wide test tube.

they attain the highest point of the bend. The short limb of the syphon passes through a small rubber stopper which exactly fits the upper opening of the filter tube, and in which it is so adjusted that 20 mm. of the syphon project. When the apparatus is assembled, therefore, the end of the syphon within the filter tube is situated in the middle of the upper widened portion, thus causing the transferred liquid to fall straight on to the filtering layer without being deflected to the walls. The syphon must always be repeatedly cleaned with sulphuric-chromic acid and water before use.

The test tube with the precipitate of silver halide is placed

in an empty beaker ; the long limb of the syphon, which has already been placed in position in the filter tube, is introduced into the test tube and is lowered, by adjusting the wide tube in the rubber stopper of the filter flask, until its lower end almost reaches the precipitate at the bottom of the test tube. The pump is then turned on to such an extent that about 2 drops of filtrate pass per second. After the bulk of the liquid has thus been removed, the inner surface of the test tube is rinsed with dilute nitric acid (1 : 100) from a small wash-bottle with a fine jet, and the test tube is shaken in order to wash the precipitate energetically ; this is very easily carried out by lifting the filter flask slightly with one hand, and shaking the test tube with the other. The whole filtering apparatus is then lowered so far that the end of the long arm of the syphon reaches to the bottom of the test tube. The suspended precipitate is thus transferred to the filtering surface in a very short time, and any further residue is transferred by further rinsing, which is carried out by directing the stream from the wash-bottle tangentially to the inner wall of the test tube and simultaneously rotating this latter about its axis. The empty test tube, the sides of which are still moist, is then rinsed in the manner already described with alcohol, which is also introduced in a thin stream whilst rotating the tube. Every one who carries out this operation for the first time will be surprised to find that further quantities of silver halide collect in the apparently clean tube in consequence of the surface tension phenomena due to the interaction of the two liquids ; these further quantities of silver halide collect, fall to the bottom and are sucked over by the vacuum on to the filter. The test tube is then twice rinsed alternately with dilute nitric acid and alcohol in order to ensure that the whole precipitate is quantitatively transferred to the filter. It is only necessary to loosen adherent particles of precipitate with the " feather " and a jet of dilute nitric acid and alcohol in exceptional cases, due to unsuitable heating of the test tube in the water bath or failure to close it, thus causing small particles to dry on to the walls.

When filtration is finished the syphon is removed from the filter tube with the rubber stopper, the portion of the syphon projecting into the tube is rinsed with alcohol and the filter tube filled with alcohol up to the rim. After this has passed through the filter, the latter is removed from the outer glass tubing, the rubber collar removed from its stem, and it is carefully wiped with a clean cloth, and then connected with the pump by means

of a suitable glass connecting piece as already described. After fitting it with an air filter it is dried in the regenerating block, and once more weighed after careful wiping and after having been allowed to stand for the necessary period.

The Course of the Determination of Sulphur

The microanalytical determination of sulphur has so much in common with the determination of halogens, which has just been described, that it will be quite sufficient to describe the features by which they differ.

The bead-tube is filled with perhydrol which has been diluted with five times its volume of water. The function of the perhydrol is to convert any lower oxides of sulphur which may have been formed into sulphuric acid and Caro's acid. After charging the tube the combustion of the organic matter is carried out exactly as has been described in detail in connection with the halogen determinations, the only difference being that a slower current of oxygen is employed of two to three bubbles in two seconds, and, consequently, the movable burner is moved forward with more care ; the reason for this difference is that, as is well known, sulphur trioxide is only absorbed quantitatively by aqueous liquids after a long period of contact.

After the combustion is completed and the boat and both platinum contacts have been removed, the tube, which is clamped at an oblique angle, is then rinsed into a platinum dish with polished inner surface, and of a capacity of 50 to 60 c.c. ; otherwise the rinsing takes place in the same manner as in the halogen determination, the only difference being that in this case neither sulphite nor any other reagent is previously introduced into the tube. Finally, the wide test tube, by which the pointed end of the combustion tube was protected during the combustion, is twice rinsed with a little water and these washings are united to those already in the platinum dish.

During our first experiments, and until 1913, we did not use a platinum dish for the further treatment of the washings, but used instead flat-bottomed glass dishes, until one day all the sulphur determinations gave values which were too high by several per cent. This was caused by the use of a new dish, which gave rise to the separation of silica during subsequent operations, thus causing a positive error in the weight of the barium sulphate precipitate. This error can be avoided by steaming the glass dishes for several hours on the boiling water bath, but for several

years we have found it preferable to use platinum dishes only, although small quantities of precipitate are less easily visible in these than in glass dishes. Moreover, the recovery of small invisible particles is so efficiently achieved with the help of the surface tension phenomena between aqueous liquids and alcohol that the visibility of the precipitate is really quite unimportant.

To the united washings in the platinum dish a freshly prepared mixture of 1 c.c. of barium chloride solution (1 : 10) and 5 to 10 drops of dilute hydrochloric acid, which has been tested for perfect clearness, is added ; the dish is then covered with a clean watch-glass with the convex side downwards, and is heated on the water bath. After a few minutes the bulk of the perhydrol is decomposed by the catalytic action of the platinum surface, and increasing quantities of barium sulphate gradually separate. It is desirable and convenient to diminish the volume of the liquid before filtration. This is effected by evaporation on the water bath, with protection from dust ; in the ordinary way this requires about one hour, but is preferably hastened by aspirating over the surface a stream of air carefully filtered through cotton-wool with the aid of a small water pump. When the volume has decreased to 3 to 4 c.c. the platinum dish is once more covered with the watch-glass and placed in a vessel of cold water in which it is left for 10 to 15 minutes in order to cool completely, after which the barium sulphate precipitate may be filtered.

Many attempts to transfer the barium sulphate precipitate to the filter by a similar method to that which is so successful with the silver halide precipitates failed on account of the high specific gravity of the precipitate. Moreover, as I demonstrated in 1910, it is necessary to ignite this precipitate in order to obtain it free from barium chloride by washing once only, and this operation is impossible with the present form of filter tube.

For these reasons I have continued to use the same apparatus which I used before 1912. This consists of the micro-Neubauer crucible supplied by the firm of Heraeus, of Hanau, which is fitted with a lid and a capsule fitting over the lower portion. The crucible itself is 14 mm. in height, and has a diameter of 12 mm. above and 10 mm. below. The filtering layer consists of compressed iridio-platinum sponge which completely retains barium sulphate precipitated in the cold at a comparatively high rate of filtration.

During filtration the crucible, naturally without the lower cover, is placed on the glass tube *G*, of about 10 mm. outer

diameter, that is, of the same diameter as the bottom of the crucible, and is held in position by the rubber sleeve *M*, half of which is drawn over the tube ; the other projecting half serves as an air-tight connection when the crucible is pushed into it, particularly if it is first moistened. The glass tube is held in a perforated rubber stopper, which is in turn inserted into a small filtering flask. As the filtration of barium sulphate must be carried out with slight suction only, I have never used the pump, but evacuate the flask by suction by the mouth with the help of a rubber tube 50 cm. long, connected with the side tube of the flask and provided with a pinchcock, as I find this to be the most successful procedure.

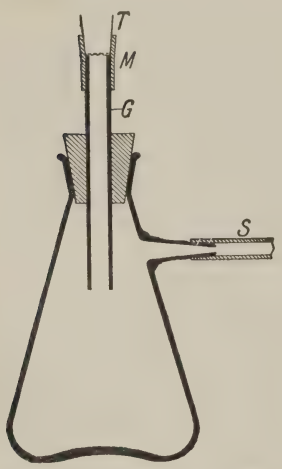

FIG. 27.—Apparatus for filtration of barium sulphate. ($\frac{1}{2}$ actual size.)

T. Micro-Neubauer crucible. *M*. Rubber sleeve. *G*. Adjustable glass tube of 10 mm. outer diameter. *S*. Rubber tubing, 50 cm. long with glass mouth-piece and pinchcock at end not shown in figure.

Before each analysis the inside of the crucible is cleaned under the tap with a small wad of cotton-wool wound round a match, and is then repeatedly washed with water after inserting it into the moistened rubber sleeve of the filtering apparatus. Very occasionally only, and after use for a long time, it is necessary to remove particles of barium sulphate held in the filtering layer by means of a little warm concentrated sulphuric acid. After a preliminary washing by one or other of the methods indicated the crucible is finally washed with distilled water, removed from the sleeve with three fingers and closed with the lid and lower cap, and is then best placed on the lid of a fairly large platinum crucible of not less than 30 mm. diameter and dried with a small flame, during which operation it often oscillates in consequence of the formation of steam ; it is then ignited at a dull red heat by increasing the flame. The lid may also be held in the flame with clean platinum-tipped forceps in order to heat it to redness.

After removing the flame the crucible is allowed to stand until it and the support have cooled to not more than 150° C. It is then seized with the platinum-tipped forceps in one hand whilst, with the other, a copper block is placed near it, after which, the crucible, with cap and lid, is placed on the copper block and transferred with this to the desiccator.

The time which must elapse before weighing may be appreciably shortened by transferring the crucible to a second cold copper block after the first few minutes, thus increasing the rate of cooling. By proceeding in this manner it is possible to weigh the crucible after not more than ten minutes. The crucible is always transferred to the balance by first placing crucible and copper block near the latter, then seizing the crucible by its centre with the forceps, which are held in the left hand, and placing it on the pan. At the first attempts with an unpractised hand the crucible may fall over, but after a little practice this operation is easily controlled.

One should never omit to remove the copper block from the balance case before the actual weighing, as even very slight temperature differences between this mass of metal of comparatively high heat capacity and the interior of the balance case may lead to a deflection of the zero point.

The weighed crucible is returned to the copper block, which latter is placed in the desiccator, and is then transferred to the spot where the barium sulphate precipitate is to be filtered. For this purpose the lid and lower cap are removed from the crucible, during which operation it is handled very gently, and it is then placed in the moistened rubber sleeve of the filtration apparatus and moistened internally with a drop of distilled water, after which a permanent vacuum is obtainable by suction with open pinchcock, and by then closing the latter ; if the filtering layer is not moistened, air has unhindered access.

For the transference of the barium sulphate precipitate from the dish into the micro-Neubauer crucible a small instrument is essential which I described in my original publication. This is the " Feather " (Fig. 28), which consists of a glass capillary of 1 to 1·5 mm. external diameter, and about 120 to 150 mm. long, to the open end of which a small feather is cemented. After some trouble I found that small snipe feathers, which are commonly used by sportsmen for adorning their hats, and also by artists for certain operations in painting, are suitable for the purpose. These are very resistant and stiff, and are also of a suitable shape which does not require modification by trimming. The smaller specimens are preferable. As a cement for the feather, the glass cement is used which has already been described in connection with the closing of the absorption tubes in the determination of carbon and hydrogen ; a small piece of this is placed in the open end of the capillary, and melted by warming on a pre-heated piece of metal, but not in the flame ; a portion of the molten cement

Fig. 28.—The feather. (Actual size.)

then enters the capillary, the end of which is still kept warm whilst the shaft of the feather is inserted. When the operation is unsatisfactorily carried out a cavity remains between the glass wall of the capillary and the shaft of the feather which might retain particles of the precipitate. The excess of cement which adheres outside the capillary is removed mechanically while still warm, and the feather is then washed quickly and consecutively in benzene and alcohol, and finally in a little ammoniacal soap solution, by carefully rubbing it between the fingers. It is always kept under cover in a stoppered test tube.

The barium sulphate precipitate settles at the bottom of the platinum dish, and in order to transfer it to the filter the dish is held in the left hand whilst the right holds the feather, which has previously been well rinsed with alcohol and water, vertically above the centre of the mouth of the crucible. The liquid contents of the dish are then poured down the feather into the crucible, without disturbing the precipitate, until the crucible is full, after which it is refilled in the same manner. The following precautions should be observed during this somewhat delicate operation :

1. It is advantageous to rub the edge of the dish, at the point of pouring, which need not necessarily be the lip, with the finger, which has been slightly greased by rubbing it on the head or the side of the nose.

2. During decantation both elbows should be held close to the body, so that the edge of the dish and the feather are always maintained in contact above the centre of the mouth of the crucible.

3. The top of the liquid in the crucible should never be touched with the point of the feather, as in that case precipitate already in the crucible would creep back up the feather.

After the liquid has been disposed of as described, the rim of the dish is rinsed with a fine stream of water (1 to 2 c.c.), and the precipitate is stirred up with the point of the feather, and at once transferred to the empty crucible. The inner surface of the dish is again rinsed with water, from the edge inwards, and is then rubbed with the feather from the edge to the centre, and the contents once

more transferred to the crucible. The whole circumference of the inner surface of the dish is then rinsed with alcohol in a continuous stream, and the liquid once more transferred to the crucible down the feather, which is held in a vertical position. After rinsing with alcohol the crucible is again rinsed as before with water, and the transference of any visible particles of precipitate assisted by rubbing with the feather. The rinsing of the interior of the dish with alcohol and water is repeated, at least once in the manner just described. With a little practice the last residues will be removed from the dish by the double utilisation of the surface tension between alcohol and water. The beginner will find that a rather considerable time is needed. It is always necessary to use water for the final washing, for, if the precipitate and the filter layer are left moistened with alcohol, violent spirting of the precipitate may occur on subsequent heating, which may even damage the filtering layer.

The crucible is now gently removed from the rubber sleeve, wiped outside with a clean cloth, fitted with its lid and lower cover, and placed as above described on to the large platinum lid, where it is once more gradually raised to a dull red temperature. After cooling and removing the lid and lower cap it is once more inserted in the moistened rubber sleeve, filled up with water, to which a drop of dilute sulphuric acid has been added, and filtered by suction with the mouth. After the liquid has all passed through the filter, water is passed through once more, and possibly a third time ; it is then fitted with lid and lower cap, placed on the platinum support, and once more carefully raised to a dull red heat.

The precautions which have just been mentioned are absolutely necessary, for as I showed during the latter portion of 1910, in Innsbruck, any barium chloride which is carried down with the barium sulphate precipitate can only be removed by washing after the precipitate has been ignited ; I was able to demonstrate at that time that ignition of the crucible should never be carried out with a direct flame, but always through a fairly thick platinum support, as the thin walls of the crucible when red hot are so permeable to the reducing gases of the non-luminous flame that barium sulphate is reduced to barium sulphide, which goes into solution on washing, and thus causes loss.

The ignited crucible is transferred to the balance in the manner already described, and is weighed after cooling. It is advisable to control the weight by once more washing, igniting and weighing. Experience shows that with pure barium sulphate this

results in a loss of weight of 0·005 mgm. only, or at most 0·01 mgm. if large quantities of water are used. It is, therefore, superfluous to weigh the barium sulphate precipitate to a greater accuracy than 0·005 mgm.

The Determination of Sulphur by Titration in Substances free from Nitrogen and Halogens

The determination of sulphur in any substance containing neither nitrogen nor halogens can be carried out in a very simple manner ; unfortunately it has not so far proved possible to apply the method to substances containing halogen or nitrogen, in spite of many attempts to do so. In carrying out the process it is important to use perhydrol solution of neutral reaction. According to my experience Merck's perhydrol, taken from a fresh bottle, always shows neutral reaction with Methyl Red as indicator, but on keeping for several weeks or months an acid action is obtained, and, therefore, the dilute solution of perhydrol (1 to 5) which is used in carrying out determinations of sulphur by titration must be neutralised in the wide test tube, before being introduced into the bead tube, by adding sodium hydroxide solution, drop by drop, in presence of a trace of the neutral indicator (page 97) until a neutral canary-yellow tint is obtained in the liquid.

After completing the combustion the bead tube is rinsed with pure water by filling and emptying it two or three times as already described, and the washings are collected in a Jena glass conical flask, and then titrated with standard sodium hydroxide solution until the neutral canary-yellow tint is obtained. During this titration, as in all titrations using Methyl Red as indicator, account must be taken of the fact that this indicator shows the phenomenon of subsequently turning pink. (That is, there is a time lag to the reaction.—*Translator.*) It is, therefore, necessary to refrain from taking the final reading until the canary-yellow tint has been maintained for two minutes. For simplicity, the N/70 sodium hydroxide solution used for the micro-Kjeldahl determination may be replaced in the titrimetric determination of sulphur by an N/100 solution.

Some Analytical Results

(*a*) Halogen Determinations

Tribromphenol :

5·040 mgm. :	8·545 mgm. AgBr. .	= 72·15 per cent. Br.
	Theory	= 72·27 per cent. Br.

Trichlordinitrobenzene :

6·558 mgm. : 10·41 mgm. AgCl. . = 39·27 per cent. Cl.
Theory . . . . . = 39·19 per cent. Cl.

2-Chloranthraquinone-5-Carboxylic Acid :

6·618 mgm. : 3·34 mgm. AgCl. . . = 12·48 per cent. Cl.
Theory . . . . . = 12·38 per cent. Cl.

7-Chlor-1·9-Benzanthrone :

7·739 mgm. : 4·22 mgm. AgCl. . . = 13·49 per cent. Cl.
Theory . . . . . = 13·40 per cent. Cl.

(*b*) Sulphur Determinations

Gravimetric

Sulphonal :

6·185 mgm. : 12·745 mgm. $BaSO_4$. = 28·31 per cent. S.
Theory . . . . . = 28·10 per cent. S.

Trional :

6·148 mgm. : 11·925 mgm. $BaSO_4$. = 26·65 per cent. S.
Theory . . . . . = 26·48 per cent. S.

Sulphanilic Acid :

5·680 mgm. : 7·60 mgm. $BaSO_4$. = 18·38 per cent. S.
Theory . . . . . = 18·25 per cent. S.

Volumetric

Sulphonal :

5·103 mgm. : 6·27 c.c. N/70 NaOH . = 28·15 per cent. S.
Theory . . . . . = 28·10 per cent. S.

Dithiohydroquinone ($C_6H_4(SH)_2$) :

5·492 mgm. : 10·83 c.c. N/70 NaOH . = 45·16 per cent. S.
Theory . . . . . = 45·11 per cent. S.

Methylmercapto-1-di-(acetylmercapto)-2-4-benzene ($C_6H_3.SCH_3.(SCOCH_3)_2$) :

5 363 mgm. : 8·28 c.c. N/70 NaOH . = 35·36 per cent. S.
Theory . . . . . = 35·33 per cent. S.

The Determination of Sulphur and of Halogens in small Quantities of Material by Carius' Method

Although the determination of these elements by the methods previously discussed is undoubtedly preferable, I will also describe their determination by the method of Carius for the sake of completeness.

The substance is weighed in capillaries, 1 to 1·5 mm. wide and 30 mm. long, which are open at both ends. Both the empty and

the full capillaries are weighed in a horizontal position on a stand of aluminium wire. By pressing the empty weighed capillary into the substance contained in a watch-glass, this may be introduced to a length of 2 to 4 mm. If necessary, the substance is forced into the capillary by means of a tightly-fitting glass thread in order to make room for more. The end from which the capillary has been filled is carefully brushed and wiped before weighing, so that no loss may occur during that operation. It is also advisable to allow that end to project over the rim of the balance pan.

The organic substance is decomposed in small pressure tubes of soft glass, of about 10 mm. external diameter and 200 mm. long. For halogen determinations these are charged with a lump of silver nitrate of the size of a hemp seed, and for sulphur determinations with a lump of barium chloride of similar size. The capillary with the weighed material is then introduced and, finally, 0·5 to 1 c.c. of concentrated nitric acid is added, which is allowed to run down the wall in order to wash down any adhering material. The tube is closed with the production of a long, thick-walled capillary which is particularly easily effected in tubes of this small size, and the sealed end is allowed to cool in a smoky flame. The tubes can be heated in any tube furnace. Franz X. Eigner, mechanic at the Innsbruck Physiological Institute, who has repeatedly been mentioned, built a small tube furnace for me of suitable size for the tubes described, and this can be highly recommended. It offers the advantages, not only that the necessary temperature can rapidly be attained, but also that after the operation is finished the furnace can rapidly be cooled. This apparatus is also particularly suitable for preliminary experiments in sealed tubes for preparative purposes. It is now built by H. Anton Orthofer, the mechanic at the Institute for Medicinal Chemistry at Graz University.[1] After cooling, the capillaries of the sealed tubes are opened; this is best done by cutting off the point with a glass-cutter. After the outer surface has been carefully cleaned the upper third of the tube is removed in this manner. After cutting, glass splinters are carefully removed by wiping, and the cut is then touched with a red hot drop of glass. If this operation is carried out whilst the tube is in a very inclined position, for instance resting in a dish, the introduction of glass splinters into the interior is avoided.

[1] Quite recently, Friedrich Holtz has recommended a bath of methyldiphenylamine (b.p. 295° C.) for heating sealed tubes. (*Ber.* (1922), **55**, 1496.)

The separate upper portion is rinsed with hot water whilst inverting it in the mouth of the lower portion of the tube, the latter being held in a test tube stand meanwhile. After removing the upper portion, the contents of the lower portion of the tube are rinsed into a clean glass dish of about 70 c.c. capacity with a fine jet of water directed upwards into its interior. The capillary falls into the dish with the precipitate which has been produced, and is seized with clean platinum-tipped forceps and rinsed thoroughly into the dish with water. If necessary, the " feather " must be used.

For halogen determinations the precipitate is transferred to a filter tube by the method described on pages 116 and 117, with the help of the syphon and of the interfacial surface tension between alcoholic and aqueous solutions as there described. In the case of halogen determinations it is still simpler, after rinsing the separate upper portion of the tube, to insert a platinum wire, which is preferably not perfectly straight, into the bore of the capillary, and to lift the latter by this means out of the liquid. It is then rinsed within and without, so that the washings are collected in the lower portion of the tube, and the precipitate of silver halide is then drawn directly into the filtering tube by means of the syphon. It is advisable first to round off the sharp edges of the tube in the flame in order to avoid contamination with splinters.

When determining sulphur it is necessary to add a little hydrochloric acid to the contents of the dish and then to evaporate completely, and to repeat this process after further addition of hydrochloric acid in order completely to remove the nitric acid. The precipitate is collected and weighed in the micro-Neubauer crucible exactly in the manner already described.

VII. The Microanalytical Determination of Phosphorus and Arsenic in Organic Substances (described by Dr. Hans Lieb)

A. Phosphorus

The study of a microanalytical method of determining phosphorus in organic substances could be attempted with hope of success as the preliminary conditions for the work had already been evolved. An entirely satisfactory method of determining phosphoric acid in fertilisers and soils had been described by von Lorenz, depending on the direct weighing of the ammonium phosphomolybdate precipitate, and described by him as the

" method of drying the ammonium phospho-molybdate precipitate by ether." [1]

The conditions when using this method are particularly favourable in so far as the precipitate when weighed is thirty times as heavy as the phosphorus pentoxide which it contains, and sixty-eight times as heavy as the phosphorus. The precipitates need not, therefore, be weighed to an accuracy of more than at most 0·05 mgm., as if 5 mgm. of material are used for the analysis a difference of 0·1 mgm. in the precipitate corresponds to 0·04 per cent. of phosphorus only. Although the composition of the yellow ammonium phosphomolybdate is not exactly known, von Lorenz has shown experimentally that if approximately the same quantities of reagents are used, and the same methods are followed, precipitates of the same composition are always obtained, so that one may use a factor for phosphorus which is easily determined empirically.

A very convenient and satisfactory apparatus was also available for collecting and weighing the precipitate, namely, Pregl's "halogen filtering tube," which can also be used in this case, together with the automatic arrangement for transferring the precipitate by suction.

There remained to be discovered a simple method of oxidising organic substances, and of converting the phosphorus into phosphoric acid. Almost all the many methods described in the literature would have been more or less suitable, and could have been selected, according to the nature of the material, particularly those methods which depend on melting or heating the compound with the addition of oxidising materials.

In the microanalytical determination of phosphorus in the few substances which were available in a state of absolute purity, it was found that quite satisfactory results were obtained by mixing them with sodium carbonate and saltpetre in a platinum boat, covering with the same material and then heating in a stream of oxygen.

The phosphoric acid factor is determined by using salts, the exact phosphoric acid contents of which are known. Lorenz used for this purpose primary ammonium phosphate $(NH_4)H_2PO_4$ and primary potassium phosphate KH_2PO_4. "These two salts crystallise free from water, can be obtained in a perfectly pure condition by recrystallising, and may then be dried for an

[1] The Agricultural Testing Station, 1901, pp. 183–200. See *Zts. f. analytische Chemie* (1907), **46**, p. 192. Detailed description in *Zts. f. analytische Chemie* (1912), **51**, p. 161.

indefinite time at 110° C." For the following experiments secondary sodium ammonium phosphate $(NH_4)NaHPO_4 . 4H_2O$ (209·31) containing 33·92 per cent. of P_2O_5 was used ; this material crystallises with water of crystallisation, but can be obtained in the pure condition by repeated recrystallisation. Under the conditions which will be described below, a series of analyses showed a somewhat higher phosphoric acid factor than 0·03295, the figure given by Lorenz.

We will here give the results of a series of analyses, in which quantities of phosphoric pentoxide were used, which correspond with the amount of phosporus which would be present in the analysis of organic substances :

Quantity of $(NH_4)NaHPO_4$, $4H_2O$ in mgm.	Corresponding quantity of P_2O_5 in mgm.	Weight of ammonium phosphomolybdate obtained in mgm.	The calculated percentage of P_2O_5 in the yellow precipitate.
2·595	0·8803	26·42	3·332
3·643	1·236	37·11	3·330
3·700	1·255	37·74	3·326
4·837	1·641	49·43	3·319
5·052	1·705	51·48	3·311
6·562	2·226	66·70	3·337

The arithmetical mean of the six figures in the last column is 3·3260, a figure which gives the percentage of P_2O_5 in the precipitate of ammonium phosphomolybdate. This corresponds to a phosphoric acid factor of 0·03326, and a corresponding phosphorus factor of 0·014524, which represents the figure by which the weight of the yellow precipitate obtained must be multiplied in order to obtain the weight of phosphorus present in the material analysed. The accuracy of this factor is confirmed by the results of the analysis of two absolutely pure organic preparations containing phosphorus which will be given at the end of this section. It follows from the value of this factor that the precipitate of phosphomolybdate, after drying with ether and in vacuo, has approximately the composition $(NH_4)_3PO_4 . 14\ MoO_3$, but that a very small amount of the material remains in solution. The unusual circumstance that a precipitate is weighed, in this case, which is roughly 69 times as heavy as the element to be determined, renders it unnecessary to use the microchemical balance

excepting for the material to be analysed, whilst for weighing the yellow precipitate any balance may be used which weighs with an accuracy of 0·1 mgm. The reagents needed for the determinations were prepared exactly according to the instructions of Lorenz, which may, however, be repeated here.[1]

1. *Sulphate-Molybdate Reagent.*—50 gm. of ammonium sulphate are dissolved in 500 c.c. of nitric acid of specific gravity 1·36 in a litre flask. 150 gm. of powdered ammonium molybdate are treated with 400 c.c. of boiling water in a porcelain dish and stirred until solution is complete. The solution is rinsed into a flask with a little water, cooled to the temperature of the room, and poured in a thin stream with stirring into the nitric acid solution of ammonium sulphate. The resultant liquid is diluted to 1 litre, allowed to stand for two days, filtered and kept in a well-stoppered bottle of brown glass in a dark, cool place.

2. *Nitric Acid containing Sulphuric Acid.*—30 c.c. of sulphuric acid of sp. gr. 1·84 are poured into 1 litre of nitric acid of sp. gr. 1·19 to 1·21. The latter acid is obtained by mixing 357 c.c. of nitric acid of sp. gr. 1·40 with 500 c.c. of water.

3. *A 2 per cent. Aqueous Solution of Pure Ammonium Nitrate.*—Should the solution not be weakly acid it must be acidified with a few drops of nitric acid per litre.

4. *Alcohol of 90 to 95 Volume per cent.*—This should leave no residue on evaporation and should show no alkaline reaction.

5. *Ether.*—This should leave no residue on evaporation, should show no alkaline reaction and should not contain too much water. 150 c.c. of this ether should dissolve 1 c.c. of water at room temperature to form a perfectly clear solution.

6. *Commercial Acetone Purissimum.*—This must show neutral reaction, must contain no fraction boiling above 60° C. and must be free from aldehyde. It must be kept in brown glass bottles.

7. Purest, finely-powdered, calcined sodium carbonate and purest, finely-powdered potassium nitrate mixed in the proportion 1 to 1.

Heating in a stream of oxygen is carried out in a long Jena combustion tube about 15 cm. long, one end of which is drawn out to a wide capillary and bent upwards at right angles. In order to carry out a determination, 2 to 5 mgm. of the substance to be analysed is usually weighed into a platinum boat, such as is used for the micro-determination of carbon and hydrogen. An excess of the finely-powdered mixture of sodium carbonate and

[1] Exact description in *Zts. f. analytische Chemie* (1912), **51**, p. 168.

saltpetre is added, the whole mixed with a short platinum wire with a loop, which is finally laid on the boat, and then carefully covered with the oxidising mixture. The boat is then placed in the combustion tube, a slow stream of oxygen is passed and the boat is carefully heated, passing the burner from the front to the back in a direction opposite to that of the gas current.

As soon as the reaction is finished, that portion of the tube containing the boat is heated for some minutes with a full, very hot Bunsen flame, and is then allowed to cool in the stream of oxygen. The boat is then boiled out with dilute nitric acid in a small test tube and the solution poured through a filter into the precipitating vessel, which has previously been carefully cleaned with sulphuric-chromic acid. This consists of a wide thick-walled test tube, such as is used for the micro-halogen determinations. Should particles of the melt have spirted into the combustion tube during the reaction, this must also be rinsed out with hot dilute nitric acid. The drawn-out portion of the combustion tube is bent at right angles for this purpose, as by holding the tube obliquely it is easy to dissolve the attached material and to allow the solution which is obtained to drain out through the capillary. To the clear filtrate 2 c.c. of nitric acid containing sulphuric acid are added, and also water, if necessary, to dilute the total volume to 15 c.c. The liquid is then warmed on the boiling water bath. The hot solution is well shaken, 15 c.c. of the sulphate-molybdate reagent poured into the middle of it, the vessel then allowed to stand for three minutes, and again shaken for half a minute. The precipitate is then allowed to settle for at least one hour, and then filtered on the pump. The filter tube to be used for this purpose has previously been cleaned and dried by dissolving any ammonium phosphomolybdate on the filter in ammonia, washing with water, hot dilute nitric acid, and again with water, and finally displacing the water by alcohol and ether or by acetone. The filter tube is then wiped with moist flannel and dry chamois leather, and left for at least half an hour in a desiccator containing no drying agent which is evacuated to the utmost extent with an ordinary water pump. In no case should the pressure in the desiccator be more than 150 mm. of mercury. The disappearance of all smell of ether or acetone is an indication that the tube when removed from the vacuum is ready for weighing.[1]

The filter tube is removed from the desiccator immediately before

[1] According to Neubauer and Lücker (*Zts. f. analytische Chemie* (1912), **51**, p. 164), acetone is better than ether for removing the last portions of water.

use, placed on the balance, and the time carefully noted which has elapsed between removal from the desiccator and the final weighing, which may conveniently be 5 minutes. These conditions must, of course, be exactly repeated when the precipitate is weighed. The necessary time is considerably shortened in this way, as otherwise one would have to wait 30 minutes until the filter tube showed a constant weight, on account of the hygroscopicity of the asbestos layer. Rapid work is also advantageous on account of the somewhat hygroscopic character of the yellow precipitate.

The precipitate is drawn into the filter tube by means of the same automatic arrangement as is used for the silver halide precipitate. After the supernatant liquid has been syphoned from the precipitate it is washed in the test tube with the 2 per cent. ammonium nitrate solution, and afterwards transferred to the filter ; in order to remove the last traces of precipitate from the walls of the precipitating vessel 95 per cent. alcohol and ammonium nitrate solution are used alternately. The tube is finally filled once with alcohol, and then twice with ether or acetone, in order to displace the alcohol. The filter tube is then wiped with moist flannel and dry chamois leather and placed in the desiccator, which, as already mentioned, must contain no drying agent. After drying for half an hour in vacuo the tube is weighed under exactly the same conditions as those of the initial weighing. It may once more be mentioned that this weighing of the precipitate can be carried out on an ordinary analytical balance with an exactitude of 0·1 mgm. without affecting the accuracy of the result, on account of the exceptional circumstance that a determination of the weight of the molybdate precipitate with the ordinary macroanalytical accuracy is quite sufficient for the microanalytical determination of phosphorus.

This method of determining phosphorus is better than any other with regard to simplicity, accuracy and quickness. Scarcely more than 45 minutes are required for a determination, and if the working conditions are exactly followed the analytical results correspond within 0·1 per cent. The only difficulty which we experienced in working out the method was not of an experimental character, but arose from the fact that it was not easy to find substances of reliable purity on which to experiment.

Among the investigations on various compounds we may mention several analyses of two absolutely pure preparations

which were very kindly placed at our disposal by the late Professor Emil Fischer.

(*a*) *Diphthaloyl-phosphoric acid ester*

1. 5·952 mgm. yielded 36·08 mgm. of ammonium phosphomolybdate.
2. 5·165 mgm. yielded 31·60 mgm. of ammonium phosphomolybdate.
3. 1·408 mgm. yielded 8·55 mgm. of ammonium phosphomolybdate.

Theory for $C_{20}H_{13}PO_4$ (mol. wt. 348·1) : P = 8·91 per cent.
Found : P = 8·81 per cent. ; 8·89 per cent. ; 8·82 per cent.

(*b*) *Theophyllinglucoside-phosphoric acid*

1. 5·645 mgm. yielded 27·17 mgm. of ammonium phosphomolybdate.
2. 5·525 mgm. yielded 26·16 mgm. of ammonium phosphomolybdate.

Theory for $C_{13}H_{16}O_7N_4PO_2H + 2\ H_2O$ (440·21) : P = 7·04 per cent.
Found : P = 6·99 per cent. ; 6·88 per cent.

B. The Microanalytical Determination of Arsenic[1]

Arsenic can be quantitatively transformed into arsenic acid by oxidation of the organic material with nitric acid in a sealed tube. The arsenic acid is then precipitated with magnesia mixture as magnesium ammonium arsenate, filtered in a Neubauer crucible, and weighed as magnesium pyroarsenate after ignition.

5 to 10 mgm. of the substance to be analysed are filled into a capillary 30 to 40 mm. long, open at both ends, and of 1 to 2 mm. bore, which has previously been weighed with an accuracy of 0·002 mgm., by scooping up the finely-divided material from a watch glass and driving it home with a glass thread if necessary. When sufficient material has been pressed into the capillary this is tapped and well wiped, and then weighed again to within 0·001 mgm. The charged and weighed capillary is then seized with the forceps and dropped fairly vertically into the reaction tube which has previously been prepared.

This is made from carefully cleaned and washed soft or hard

[1] From " Abderhalden's Handbuch der Biochemischen Arbeitsmethoden " (1919), p. 727.

glass tubing, of 19 mm. external diameter, with walls of 1·0 to 1·5 mm. thickness and 50 to 100 mm. long, by sealing the one end to an evenly rounded shape. After filling the capillary with the substance the tube is held at an oblique angle, and about 0·5 to 1 c.c. of concentrated nitric acid are added whilst it is rotated, in order to rinse down any particles which may adhere to its upper walls ; the open end is then drawn out in the blow-pipe flame to form a long capillary of uniform thickness, sealed and allowed to cool in a luminous flame.

The sealed tube is then placed in a small tube-furnace, preferably tilted, such as those which are supplied of suitable quality by the University mechanic, Anton Orthofer, of Graz, and heated for several hours to 250° to 300° C., according to the ease with which the substance is oxidised. After cooling, the tube is opened, which is best done by cutting off the point with a glass-cutter, and the upper third is then carefully separated, taking care that no glass splinters fall into the tube.

The contents of the tube are then rinsed quantitatively into a glass dish of 30 to 40 c.c. capacity. The capillary of the separated portion of the tube is also carefully rinsed and the liquid is evaporated to dryness on the water bath. The residue is dissolved in 3 to 4 c.c. of ammonia on the water bath, and treated whilst hot with 1 c.c. of magnesia mixture. (This is prepared by dissolving 5·5 gm. magnesium chloride crystals and 10·5 gm. ammonium chloride in 100 c.c. of water.) The arsenic acid is thus precipitated as magnesium ammonium arsenate. As this separates at first in the amorphous condition it must be allowed to stand for 6 hours, or sometimes for 12 hours, at room temperature until it becomes crystalline, for, when amorphous, the filtration of the precipitate is very difficult.

The precipitate is then filtered into the weighed micro-Neubauer crucible with the help of the " feather " in the manner already described in detail in connection with the determination of sulphur, washed with 3 per cent. ammonia, and the final traces of precipitate transferred in the manner already described by the alternate use of 3 per cent. ammonia and alcohol. The crucible is then removed from the filter flask, closed with the lid and lower cap, placed on the lid of a platinum crucible and strongly ignited. The residue of magnesium pyroarsenate which is thus obtained still occludes magnesium salts, and must, therefore, be washed repeatedly with very weakly ammoniacal water. After again igniting strongly the crucible is placed on a copper block, and

may be weighed after 10 minutes. Further washing usually results in a further loss of weight of 0·01 mgm. only.

The method just described, when used with reasonable care, leads to results which differ from the theoretical figures by 0·2 per cent. at most.

The results are calculated as follows :—

(The logarithm of the weight of $Mg_2As_2O_7$) + log. 0·4827 + 2 — (log. of weight of materials) = log. of percentage of arsenic.

$$\frac{2\ As}{Mg_2As_2O_7} = 0{\cdot}4827 = \text{Factor.}$$

VIII. The Determination of Metals in Salts

In the case of salts of silver, gold, copper and platinum, this determination is one of the simplest micro-analytical operations. It will seldom be carried out alone, but usually by weighing the residue in the boat after the determination of carbon and hydrogen, of halogen or of sulphur. With regard to the reliability of these determinations it may be mentioned that they are accurate in the absence of halogens. Otherwise results are obtained which are somewhat low, especially in the case of platinum, as the halogen derivatives which may be formed at an intermediate stage are volatile when heated. The determination of potassium, sodium, magnesium, calcium, barium and cobalt was studied by me at my Innsbruck Institute, in co-operation with Dr. Johann Dubsky, of Zurich, in 1912, using a whole series of salts of these metals. The method which we then used was to weigh a few milligrammes of the salt into a micro-platinum crucible, and moisten it with 2 small drops of concentrated sulphuric acid. The crucible was then placed on a fairly large platinum support, covered with its lid, which had been weighed with it, and the non-luminous flame of a small burner was directed on to the centre of the lid, raising it to a dull red heat. After 10 to 15 minutes the crucible was cooled, and its contents carefully inspected in order to see whether all the sulphuric acid had been removed. In that case the final combustion of the separated carbon was effected by carefully heating the platinum support from below with a Bunsen flame. After combustion of all the carbon the crucible was allowed to cool. In the case of potassium and sodium salts it is necessary to drop a groat-sized particle of ammonium carbonate into the crucible and then reheat, in order to effect the well-known transformation of primary into secondary sulphates. Finally, the cooled crucible is placed on a copper block in the desiccator, transferred to a

second copper block near the balance case, so that the cooling may be more rapid, and weighed after a suitable interval.

I found it advantageous later to use a somewhat different method of treatment with sulphuric acid. This consists in heating the lid of the micro-crucible momentarily from above, at intervals of three to five seconds, with a fairly large and stable ("stiff") flame or, still better, with the flame of a Mecker burner, which is held in the hand whilst the crucible stands on its platinum support. Almost invisible fumes of sulphuric acid may be observed to escape from the crucible in the intervals. In this way all the sulphuric acid is driven off in a few minutes, and the further operations are carried out as already described.

Even a beginner is able to determine sodium in sodium chloride

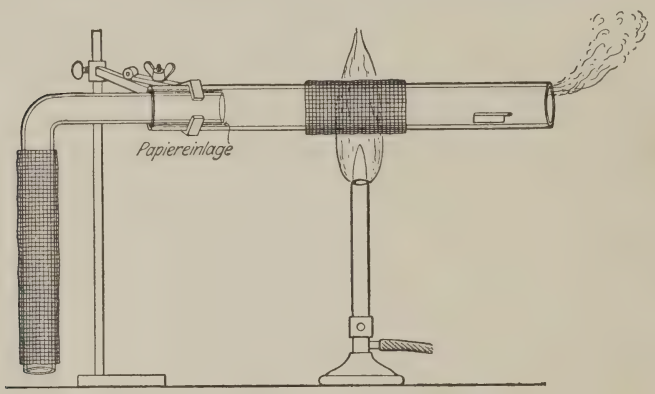

FIG. 29.—Micro-muffle. ($\frac{1}{2}$ actual size.)
"Papiereinlage" = Paper wrapping.

by this method in ten minutes, starting from the commencement of the heating process. Dr. Sernagiotto, of Bologna, carried out an analysis in my presence which was accurate to 0·2 per cent. with the smallest amount of material which anybody has ever suggested, using my microanalytical method; this was a determination of sodium as sulphate in 0·3 mgm. of sodium chloride.

In spite of these results I was always eager to discover a process which would enable such materials to be decomposed with sulphuric acid without the constant, careful supervision of the analyst. Many devices were tried from time to time until, quite recently, I discovered a device which enables the decomposition to be carried out by what might be described as an automatic method. I call the apparatus the micro-muffle. It consists (Fig. 29) of a hard glass tube, 200 mm. long and of about 15 to

17 mm. external diameter, which is clamped at one end in a horizontal position in such a manner that it can be heated from below by the hot part of a Bunsen-burner flame. The substance to be analysed is weighed into a platinum boat, treated with a drop of dilute sulphuric acid (1 : 5), and the boat inserted into the free end of the tube. In order to introduce a small drop of sulphuric acid only, by which means one avoids subsequent creeping of the material out of the boat, a capillary is used, of about 1 mm. diameter, which is drawn out at one end for some millimetres to the thickness of a hair ; this must be held vertically whilst the sulphuric acid is added.

Into the clamped end of the tube there is inserted the short limb of a tube bent at right angles, of 10 mm. outer diameter, the short limb of which is 50 mm. and the long limb about 150 mm. long. This is first covered with a short piece of rubber tubing, or with a wrapping of paper, which should not fit too tightly. The long limb is tightly covered with a roll of wire gauze about 100 mm. long. This is held in a horizontal position and heated, and then dropped to a vertical position by revolving the short limb, by which means a continuous ascending air current is produced which enters the hard glass tube and passes over the boat. This air current can be maintained indefinitely by playing the oblique flame of a Bunsen burner on to the wire gauze surrounding the long limb of the bent tube. The hard glass tube is now heated with a Bunsen flame which is just non-luminous, at a distance of about 50 mm. from the boat. It is advisable to protect this tube from the flame with a loosely-fitting roll of wire gauze about 50 mm. long. The distance of the flame from the boat is important ; if it is too large the process takes a long time, and if it is too small the contents of the boat may creep and cause loss. After the sulphuric acid has been volatilised, which is usually the case in from five to ten minutes, the flame is rapidly approached to the boat, and that portion of the tube which contains the boat is strongly heated for a further five minutes. In this way the subsequent use of ammonium carbonate becomes unnecessary, even in the determination of sodium, as the bisulphate is completely converted into the neutral sulphate by continued and intensive ignition.

The determination of lead offers considerable difficulty if one wishes to treat with sulphuric acid only in the micro-platinum crucible, as in this case reduction by the separated carbon may easily occur at points which become dry, and this would inevitably

cause loss and damage to the crucible. This may be avoided by adding a drop of concentrated nitric acid to the concentrated sulphuric acid when destroying the organic matter, and adding further nitric acid, drop by drop, as long as the residue tends to become dark in colour. Finally, the sulphuric acid is completely driven off, and the residue heated carefully from below, after which the weighing is conducted in the usual manner.

We never carried out the determination of chromium in the micro-platinum crucible, but always used very small porcelain crucibles, in which we completely destroyed the organic matter by ignition, and weighed the chromium as chromic oxide. These determinations are rather tedious as these crucibles require at least half an hour to cool to constant weight.

Examples

1·097 mgm. NaCl : 1·332 Na_2SO_4 = 39·32 per cent. Na.
Theory. 39·34 per cent. Na.

Potassium-3-5-disulphobenzoate : $C_6H_3(COOH)(SO_3K)_2 + H_2O$:

4·970 mgm. : 2·32 mgm. K_2SO_4 = 20·95 per cent. K.
Theory . . . . 20·80 per cent. K.

Potassium-*m*-benzene-disulphonate $C_6H_4(SO_3K)_2 + H_2O$

6·060 mgm. : 3·165 mgm. K_2SO_4 = 23·43 per cent. K.
Theory . . . . . 23·54 per cent. K.

Barium-*m*-bromobenzenesulphonate $C_6H_4(Br)(SO_3Ba)$:

8·360 mgm. : 3·235 mgm. $BaSO_4$ = 22·77 per cent. Ba.
Theory . . . . . 22·54 per cent. Ba.

IX. Quantitative Micro-Electro-Analysis

This was studied on account of an Austrian law to the effect that preserved vegetables must not contain more than 55 mgm. of copper per kilogramme. The complexity and tediousness of the determination of copper according to the *Codex alimentarius austriacus* induced me and, more particularly, my fellow-worker, Dr. Heinrich Poda, Senior Inspector of the Laboratory for Food

Analysis at Innsbruck, to develop a simple and rapid method of determining copper. We decided that an electrolytic method would be the simplest and most convenient, as the electrolytic determination of copper ranks with the simplest and most certain analytical methods.

The maintenance of the conditions which are recognised as necessary for the quick and quantitative deposition of copper on

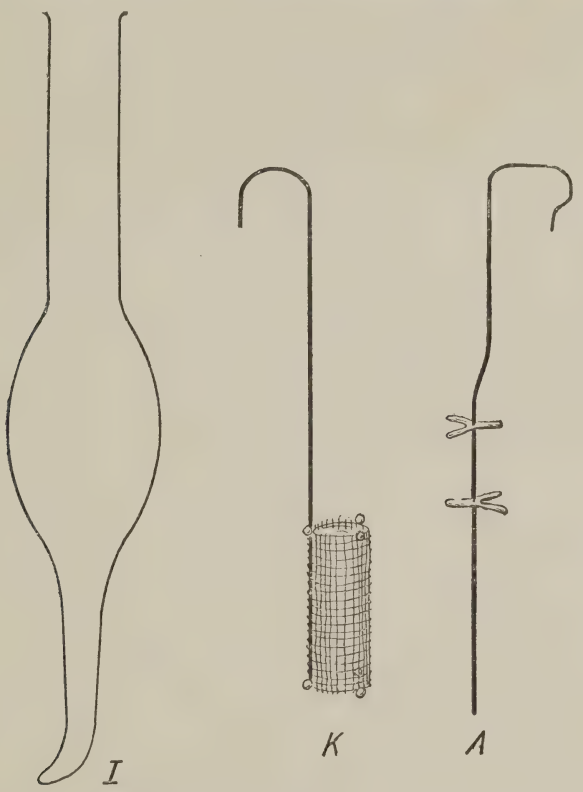

FIG. 30.—Platinum electrodes. (Actual size.) *K*. Gauze cathode. *A*. Anode. *I*. Condenser.

the cathode proved, contrary to my expectations, to be much easier than might have been expected in the separation of minimal amounts of copper, for we found that it was possible to maintain the liquid in motion by vigorous boiling instead of by the use of a stirrer, and in this way the electrolysis can be carried out appreciably more quickly ; it is necessary, however, to ensure that the process is not discontinued until the liquid has cooled completely in the closed electric circuit, as otherwise the separated copper dissolves in the dilute oxygenated sulphuric acid.

The suitability for analytical purposes of the method devised by us can be proved at any time by showing that the copper separated at the cathode, when redissolved with the help of the current and redeposited, shows an alteration of weight which is less than 0·002 to 0·005 mgm.

The most important parts of the apparatus for carrying out the process are the two electrodes. As cathode we use a platinum gauze electrode of cylindrical shape (Fig. 30, *K*), 10 mm. in diameter and 30 mm. long, as shown in the drawing. This is welded longitudinally to a stout platinum wire which projects above for a length of 100 mm. In order to avoid contact of the electrode with the wall of the containing vessel during its withdrawal, both upper and lower edges are each furnished with three glass beads, which are molten on to it and have a diameter of 1·5 mm. It should be noted that so-called " enamel " is not suitable for this purpose, as even these small quantities dissolve appreciably in the boiling liquid during electrolysis and thus cause negative errors. As anode (Fig. 30, *A*) we use a platinum wire 130 mm. long, which is bent as shown in the drawing, and to which two Y-shaped glass extensions are sealed in order to ensure that the anode takes up a definite central position within the cathode and does not touch it during withdrawal. Both the electrodes are so arranged that they may be inserted into the electrolytic cell without touching one another. The cell consists of a simple test tube, 16 mm. wide and 105 mm. long, which may conveniently be held in the arrangement shown in the drawing. This enables the cell to be readily adjusted, both vertically and laterally, whilst the terminals of the electrodes are bent over and dipped into small mercury contacts which are, in turn, connected with the current supply.

During our first experiments we found that small losses occurred through spirting of the liquid, or even through drops of liquid hanging to the wall of the empty portion of the cell. This fault can easily be overcome by placing in the mouth of the cell a loosely-fitting internal condenser with a laterally deflected tip (Figs. 30 and 31, *I*). This is made from an ordinary test tube by blowing a bulb in the middle and drawing out the closed end to a projection, about 50 mm. long, as shown in the drawing. After carefully removing any grease from its outer surface with chromic-sulphuric acid it is filled with water and used as indicated in the drawing.

As a source of current it is best to use two accumulators, and into the circuit are inserted (1) a resistance, (2) a commutator, (3) a voltmeter, as shown in the diagram of connections (Fig. 32).

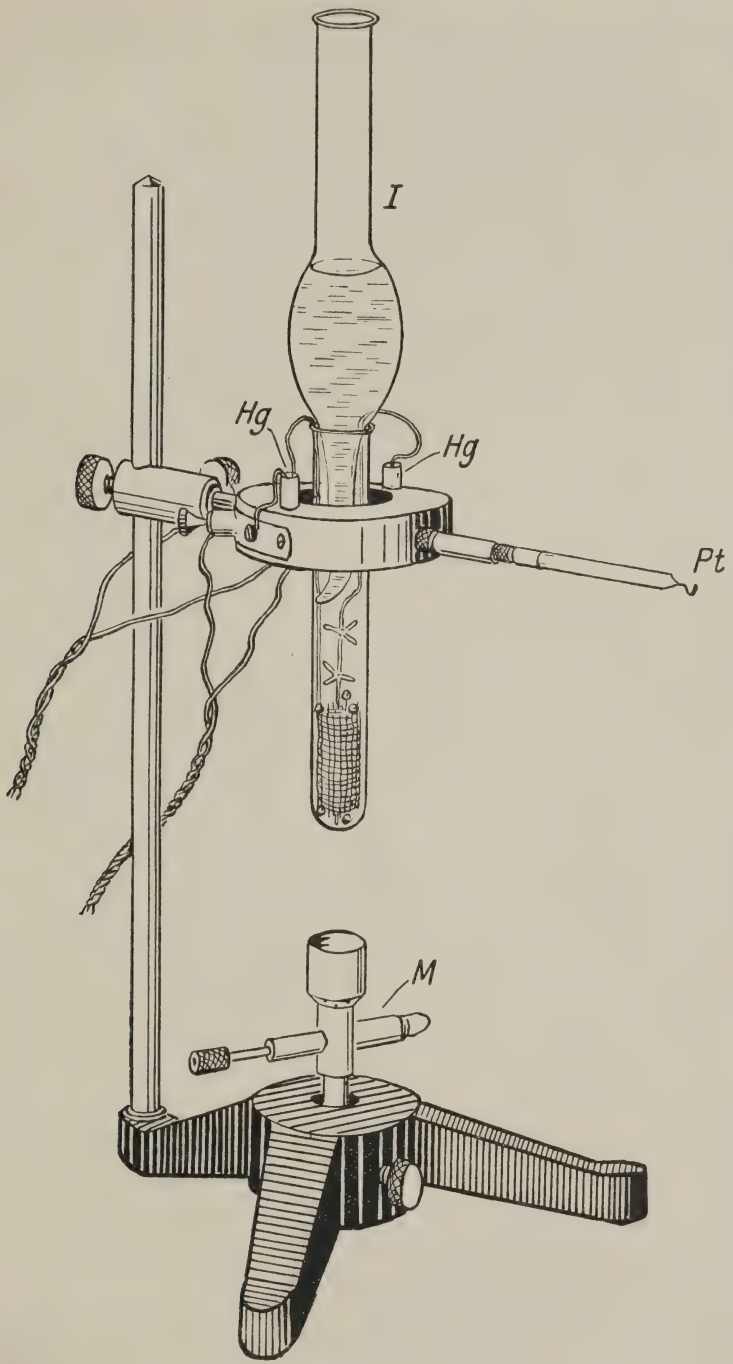

FIG. 31.—Apparatus for the electro-analytical determination of copper. ($\frac{1}{2}$ actual size.) *I.* Condenser. *Hg.* Mercury contacts. *Pt.* Platinum hook. *M.* Micro-burner.

The electrolytic determination of copper is commenced by first dipping the platinum cathode, whether covered with copper or not, successively into concentrated nitric acid, water, alcohol, and finally into pure ether, and then drying it above a Bunsen flame. The electrode can be weighed after a short interval on account of the small heat capacity and high conductivity of platinum. It is cooled whilst hanging on a platinum hook which is sealed into a glass rod attached to the micro-electrolysis apparatus (Fig. 31, *Pt*). The cathode is of convenient shape for placing on the left balance pan, on which it is supported by the three lower glass beads. The electrolytic cell and the condenser are cleaned with chromic-sulphuric acid and rinsed with water. When pouring the liquid to be analysed into the cell one should be careful that the liquid does not rise to a height of more than 35 to 40 mm. from the bottom. The weighed cathode is introduced, and then the anode, and their terminals are dipped into the corresponding mercury contacts. The electrolytic cell is then closed by inserting the condenser filled with cold water. Its tip should be in contact with the wall of the cell so that the liquid continuously flows back. After establishing the circuit the voltage is adjusted to 2 volts by means of the resistance, and the cell is heated from below with a small micro-burner. The oxygen which is evolved at the central anode prevents any superheating, and the liquid boils in a lively manner without bumping. It is advisable to slip a perforated sheet of mica over the cell at the height of the surface of the liquid in order to avoid heating of the upper portions.

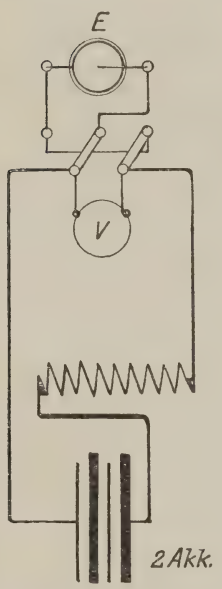

FIG. 32.—Diagram of connections.

Should the voltage alter during the analysis it must be re-adjusted to 2 volts by means of the resistance. In ten to twenty minutes one may safely assume that the last traces of copper have been deposited on the electrode. This may be confirmed by testing with ferrocyanide.

The electrolysis is completed by immersing the cell in a beaker of cold water whilst the current is maintained and using a second beaker of cold water after a few minutes. The apparatus is very convenient for this purpose, as by adjusting a single clamp the

whole cell can be transferred from the flame to the cooling water. When the contents are thoroughly cooled the condenser is removed, and, after carefully washing the hands, the anode is seized with one hand and the cathode with the other, and the anode, and then the cathode, are withdrawn, avoiding any lateral movement whilst removing them from the cell. The cathode with its copper deposit is first dipped in distilled water, than in alcohol, and, finally, in ether and dried at a considerable distance above the flame of a Bunsen burner. It is then suspended from the platinum hook and reweighed when cold.

In the determination of copper in preserved foods, which we are about to describe, the first copper deposit, obtained as above described on the electrode, is contaminated with other metals, more particularly with iron and zinc, but also with adherent traces of silicic acid. For this reason the weighed electrode is replaced in the cell, which has been rinsed in the interval, and a drop of dilute sulphuric acid and 5 c.c. of water are added ; by reversing the current the copper is completely redissolved, and is then redeposited on the cathode in the manner just described. The copper deposit which is now obtained is no longer dull and discoloured, but has the typical colour of pure copper and a bright surface. In such cases the weight of copper obtained by the second electrolysis is always smaller than that of the first deposit, and by repeating the process once more it can be shown that the second value is reproducible within 0·005 mgm.

I gather from a private communication of Mr. Benedetti-Pichler, engineer, and from experiments which he carried out in my laboratory, that he replaced the sulphuric acid in this determination by dilute nitric acid with great success. This renders it possible to dissolve considerable quantities of alloys with nitric acid, to dilute to definite volume in a large measuring flask and then to utilise 1/100 or 1/500 of the whole for analysis. A number of conditions must, however, be observed, which are :—

1. If the concentration of the nitric acid is rather too high, a crystal of potassium sulphate must be added ; and if this is not sufficient, ammonia must be introduced, drop by drop, until deposition of the copper commences.

2. A somewhat higher voltage of 2·7 to 3·1 volts must be used.

3. A single drop of alcohol must be added at the commencement of the determination in order to avoid loss by spraying due to gas evolution at the anode.

During electrolysis the cathode should project to a height of

3 to 4 mm. above the liquid, in order that one may be able to wash the walls of the cell with 1 per cent. nitric acid five minutes after starting. After a further twenty minutes electrolysis is always complete. At the commencement the electrolyte is warmed until it almost boils, and the experiment is so arranged that it cools completely during the period of electrolysis. The condenser is therefore not used.

The anode is first removed, and if it is coated with a deposit of lead peroxide this is removed by placing it in a test tube containing nitric acid to which oxalic acid has been added ; the cathode is then removed and rinsed with distilled water only. Even if the copper precipitate should be spongy it adheres to the cathode if water only is used. It was found that copper which has been deposited in a spongy condition easily becomes detached and lost if the cathode is subsequently rinsed with alcohol, in consequence of surface tension. The washed electrode is dried for a short time above a Bunsen flame, and weighed after cooling for a few minutes. The analytical results which were obtained with brass filings, gunmetal, red brass filings, bearing metal, bronze filings and metallic dust of various origin, were in good agreement with the duplicate results of macroanalysis. This process shows an undoubted advantage over electrolysis in a solution of pure sulphuric acid in so far as considerable amounts of other metals, more particularly of iron, which otherwise offers so much difficulty, do not hinder the quantitative deposition of the copper from the solution at the cathode.

The Determination of Copper in Preserved Vegetables

This is carried out by pouring the total contents of the tin into a tared wide-necked flask of such size that it is half-full, and weighing with an accuracy of 0·1 gm. Samples containing whole peas or whole beans are first broken up to a uniform paste in a large mortar. The sample is then treated with about 1/10 its weight of nitric acid of sp. gr. 1·4 and heated on the water bath with frequent shaking. After one or two hours the mass acquires a uniform pasty consistency and becomes so thin that it is easily poured out of the flask. After cooling, the whole is reweighed in order to determine the increased weight due to the nitric acid, which must be taken into account in calculating the copper contents. After thoroughly mixing, portions of 20 to 25 gm. are poured into tared test tubes, the lower ends of which are blown out to bulbs of 30 to 40 c.c. diameter, and are weighed

to 0·01 gm. The mouth of an ordinary Kjeldahl flask is slipped over the test tube and the whole quickly reversed. After the contents of the test tube have drained out of the bulb of the tube it is removed and re-weighed to within 0·01 gm.

As the success of the determination, and more especially the speed of the decomposition with sulphuric acid, depends on the exact observance of certain precautions, we will describe it in detail. The flask is first heated over the naked flame and the substance dried, as far as possible, in an air current. This is introduced by means of a bent glass tube of 10 mm. outer diameter, the longer limb of which rests on the neck of the Kjeldahl flask and ends below within the bulb. The other limb hangs vertically downwards from the end of the inclined neck and is provided with a bulb which is filled with dry cotton-wool. Over its end is slipped rubber tubing connecting it with a water blower. After the dried material has cooled, 2 to 3 c.c. of concentrated nitric acid (sp. gr. 1·4) are added, and it is again heated to dryness. After cooling, 5 to 7 c.c. of concentrated sulphuric acid are added and the whole is carefully heated to the boiling point, during which process large quantities of nitrous fumes are evolved ; on further boiling, carbon commences to separate. After cooling again, 2 to 3 c.c. of nitric acid are added, drop by drop. The solution becomes colourless on boiling, but should any colour remain the addition of nitric acid and heating are repeated.

The almost colourless contents of the flask (in the case of samples containing iron the residue remains yellow) are evaporated over a naked flame in an air current. By means of a rapid air current sulphuric acid is evaporated in this manner at the rate of 1 c.c. in one to two minutes. The whole operation, including the decomposition and boiling, takes 35 to 40 minutes if properly carried out. The residue in the flask is boiled over the naked flame for a short time with about 2 c.c. of water in order to drive off nitrous vapours which are produced by the decomposition of nitrosyl sulphuric acid, and the hot liquid is poured into the electrolytic cell through a small funnel. The remaining contents of the flask are transferred quantitatively to the cell by washing three times with 1 to 2 c.c. of hot water each time. The solution is now electrolysed regardless of any suspension of crystalline silica or gypsum. In this way filtration is avoided and the correct copper value is obtained by the second electrolysis from water slightly acidified with sulphuric acid.

Senior Inspector Dr. Poda, of the Institute for Food Analysis

at Innsbruck, has tested the suitability of the above method for the determination of the copper content of preserved vegetables in many ways.

Two separate trade samples were first analysed by him according to the method described in the " Codex Alimentarius Austriacus," namely, by incinerating with sodium carbonate dissolving in nitric acid, separating and weighing the copper as cuprous sulphide, and also by the micro-electrolytic method.

Preserved Beans

The results per kilogram of the sample were :

Copper weighed as sulphide .	37·1 ;	36 ·0 mgm.	Cu per kg.
Copper determined micro-electrolytically . . .	37·3 ;	37·9 ,,	,,

Preserved Spinach

Copper weighed as sulphide .	230·9 ;	228·3 ,,	,,
Copper determined micro-electrolytically . . .	224·8 ;	226·2 ,,	,,

Secondly, a paste prepared from fresh peas in the laboratory was treated with definite amounts of copper sulphate. The results obtained by micro-electrolysis were :

Sample 1.—Added . .			44·4 mgm.	Cu per kg.
Found . .	40·0 ;	44·8	,,	,,
Sample 2.—Added . .			78·6 ,,	,,
Found .	78·9 ;	78·4 ;	78·8 ,,	,,
Sample 3.—Added . .			100·0 ,,	,,
Found . .	99·0 ;	99·8	,,	,,

After these satisfactory results, the copper in a large number of commercial samples of preserved vegetables was determined by micro-electrolysis.

Peas (J. R. & Co., Bozen) .	39·9 ;	40·4 mgm.	Cu per kg.
Kidney beans (K. & Söhne, Aussig) . . .	39·8 ;	40·8 ,,	,,
Beans (W. H., Graz) . .	113·0 ;	113·2 ,,	,,
Salsify (W. H., Graz) . .	227·5 ;	226·2 ,,	,,
Spinach (R. B. & Co., Bregenz)	242·0 ;	245·3 ,,	,,
Spinach (R. B. & Co., Bregenz)	171·2 ;	170·5 ,,	,,
Peas (K. & Söhne, Aussig) .	33·8 ;	33·5 ,,	,,
Spinach (Ig. E., Vienna) .	107·2 ;	106·8 ,,	,,
Spinach (R. B. & Co., Aussig) .	229·5 ;	230·5 ,,	,,
Spinach (R. B. & Co., Bregenz)	316·5 ;	318·0 ,,	,,

Beans (I. G. H., Krems) .	31·1 ;	30·4 mgm.	Cu per kg.
Peas (R. B. & Co., Bregenz) .	33·7 ;	33·2 ,,	,,
Peas (P. & G., Pilcante) .	64·0 ;	64·2 ,,	,,
Spinach (I. G. H., Krems) .	32·8 ;	33·9 ,,	,,
Peas (R. B. & Co., Bregenz) .	55·8 ;	54·8 ,,	,,
Peas (R. B. & Co., Bregenz) .	50·0 ;	50·5 ,,	,,
Spinach (Ig. E., Vienna) .	47·0 ;	47·2 ,,	,,
Peas (Ldw. Gen., Mödling) .	52·5 ;	53·0 ,,	,,
Peas (K. & Söhne, Aussig) .	29·5 ;	30·5 ,,	,,

Most of the above duplicate determinations agree within 1 mgm. copper per kilogram ; this degree of accuracy is quite sufficient in the examination of foods. The method of determining ash, according to the " Codex Alimentarius Austriacus," certainly does not give any better agreement of duplicate determinations.

If 20 to 25 gm. of the product obtained by treatment with nitric and sulphuric acids are used for electrolysis the copper can be determined with an accuracy $\pm$ 0·01 mgm., that is with a maximum difference between duplicate determinations of 0·02 mgm. The quantity used for electrolysis is weighed to 0·01 mgm. only, as it is impossible to distribute uniformly the swollen masses which are less completely attacked.

It follows from the above considerations that the variation in the results expressed in milligrammes of copper per kilogram of the sample may amount to 1 mgm., which is in good agreement with the above results.

The above results in commercial samples are given in chronological order as they were received for analysis from 1912 to 1915.

It is interesting to note that the earlier years the articles supplied by most works were " coppered," and some contained considerable amounts of copper, whereas latterly a " coppered " sample has seldom been received.

The improvement is doubtless due, at any rate in great part, to the active control which has been introduced, together with the frequent intervention of the Institute of Food Analysis, as the manufacturers were forced by frequent action and penalties to keep within the legally prescribed limits.

The rapid and reliable method of determining copper by means of micro-electrolysis has contributed considerably to these results.

Finally, it may be pointed out that the determination of copper in preserved vegetables by the micro-electrolytic method just described can be carried out completely in little more than an hour by a comparatively inexperienced analyst, and the results exceed

in accuracy those obtained by the ordinary method of the "Codex Alimentarius Austriacus," which requires several days' work.

X. The Microanalytical Determination of Carboxyl

The experience gained on the titration of small amounts of ammonia during the micro-analytical determination of nitrogen by Kjeldahl's method suggested its application to the determination of the carboxyl group in small quantities of organic acids. On account of the small degree of dissociation of these acids, phenol-phthaleïn only can be used as indicator. It was found that with the use of the burettes already described, and of suitable standard alkali and acid, highly satisfactory results can be obtained with 4 to 8 mgm. of an organic acid.

The substance to be analysed is weighed out of a small weighing bottle into a small conical flask of Jena glass, of 25 to 50 c.c. capacity, as in the nitrogen determinations, and is then suspended in 2 to 3 c.c. of water or alcohol, according to the solubility of the material. The most important requisite for the process is an accurate potassium or sodium hydroxide solution of suitable strength, with which to carry out the titration. In order to avoid unnecessary calculation it is advisable to use solutions of N/45 strength, in which case 1 c.c. of the solution is equivalent to 1 mgm. of carboxyl.

The solutions are prepared from absolutely accurate solutions of N/10 HCl acid and N/10 NaOH. The latter is best prepared according to Sörensen's instructions, from a highly concentrated solution of caustic soda free from carbonate, by diluting it with carefully boiled water which has been in contact with air free from carbon dioxide during cooling. If these two N/10 solutions have been properly prepared they should be of almost exactly equal strength, both in the cold and after boiling, when titrated against one another with methyl-orange as indicator and adjusted till a neutral tint is reached. 44·4 c.c. of such a solution is introduced into a 200 c.c. measuring flask, diluted with boiled water up to the mark and the alkali coloured by adding phenol-phthaleïn to distinguish it from the acid. On then testing one solution against the other, using the burettes with 0·05 c.c. graduations, the solutions should be in complete agreement if 5 c.c. or more are used, not only after boiling, but usually in the cold. It is advisable to carry out control titrations with succinic acid which has been repeatedly recrystallised, both by boiling with excess of the

sodium hydroxide solution and titrating back, and also by direct titration with sodium hydroxide in the cold. If the solutions are correct and freshly prepared, approximately the same value will be obtained in both cases. In course of time, however, the relation between the two solutions will alter in the sense that the interval during which the red coloration persists, and also its intensity at the neutral point in the cold, becomes less. If the deviation is not large it can easily be determined with the aid of a watch and then allowed for in the course of a titration in the cold. The comparative strength of the two solutions after boiling remains constant for months. If no N/10 NaOH free from carbon dioxide is available one may proceed by diluting 60 c.c. of N/10 NaOH to 250 c.c. in a measuring flask, comparing its strength with that of an accurate N/45 HCl solution, with phenol-phthaleïn as indicator, and then correcting it accurately in a 200 c.c. measuring flask. When titrating with this solution in the cold it will be necessary to use the same duration and intensity of the red tint as was used in determining its strength. Such solutions cannot, however, be used for titration at the boiling point without further correction.

When titrating weakly dissociated organic acids it must be remembered that their salts undergo hydrolysis in aqueous solution, and that this may lead to inaccurate results. As hydrolysis is almost absent in a solution containing 50 per cent. of alcohol, one should always add the necessary quantity of alcohol during the titration. It is, perhaps, not superfluous to mention that this alcohol must previously be tested for exact neutrality, and that when carrying out this test an equal volume of water must be added, or the phenol-phthaleïn will not act as an indicator. Solid organic acids are weighed out of weighing tubes, as already described in connection with the determination of nitrogen, in quantities of 4 to 8 mgm. into a previously steamed conical flask of Jena glass of 25 to 30 c.c. capacity. If they are soluble in water, 1 c.c. of this is added, and is followed by 2 to 3 c.c. of alcohol. In the case of acids which are insoluble in water, no water need be added at this stage. The titration can be carried out in two ways. In the case of substances which are insensitive to boiling alkali N/45 NaOH is added until a distinctly red coloration is obtained, the liquid is boiled and, after cooling under the tap, is titrated back with N/10 HCl until the colour is pale pink. In cases, however, in which boiling alkali must be avoided the substance is titrated in the cold with N/45 NaOH until the

permanent pink tint is obtained. It is necessary that this titration should be carried out quickly and continuously ; it will be found that 0·01 c.c. of the solution changes the tint from colourless to a definite pink which lasts for several seconds. On waiting for a longer period this tint vanishes in consequence of the absorption of carbon dioxide from the air ; this phenomenon also occurs if older or badly prepared N/45 NaOH is used, but can be allowed for with the aid of a watch, as already described. The accuracy of such carboxyl determinations is in no way less than that obtainable with quantities of the order ordinarily used.

The calculation of the results is very simple, as 1 c.c. of the N/45 NaOH corresponds to 1 mgm. of carboxyl. Remembering that the basicity of an acid can be determined by the investigation of acid salts or acid esters, it is evident that this volumetric method of determining carboxyl is the simplest way in which the molecular weight of an acid may be determined. An example will make this clear.

We will suppose that an organic acid is found to have a carboxyl content of 45 per cent. This acid, therefore, has an equivalent weight of 100 if monobasic. If monobasic it can only form one neutral ethyl ester of molecular weight 128. If dibasic it would have double the molecular weight, and its acid ethyl ester, of molecular weight 228, would show a carboxyl value 19·7 per cent. If tribasic and of molecular weight 300, two acid esters are possible, of which the first would have a molecular weight of 356, and contain 12·6 per cent. of carboxyl, whilst the other would have a molecular weight of 328 and contain 27·4 per cent. of carboxyl. Similar calculations may be made for a tetrabasic acid of molecular weight 400. Its monobasic ethyl ester of molecular weight 428 would contain 9·3 per cent. ; its dibasic ethyl ester of molecular weight 456, 19·75 per cent. ; its tribasic ester of molecular weight 428, 41·5 per cent. of carboxyl. If, however, a carboxyl content of 15·4 per cent. were found by titration this would only agree accurately with the hypothesis that it was a pentabasic acid of molecular weight 500, two carboxyl groups of which had remained unattacked during esterification.

XI. The Microanalytical Determination of Methoxyl and Ethoxyl

The possibility of carrying out such estimations was dependent on the construction of suitable apparatus. As will be seen from the appended drawing (Fig. 33), the boiling flask *SK*, of about

3 to 4 c.c. capacity, is furnished with a side tube *A*, for the introduction of the material and of carbon dioxide. In order to prevent escape of vapours during the determination the effective cross-section of this tube is reduced by the insertion of a loosely-

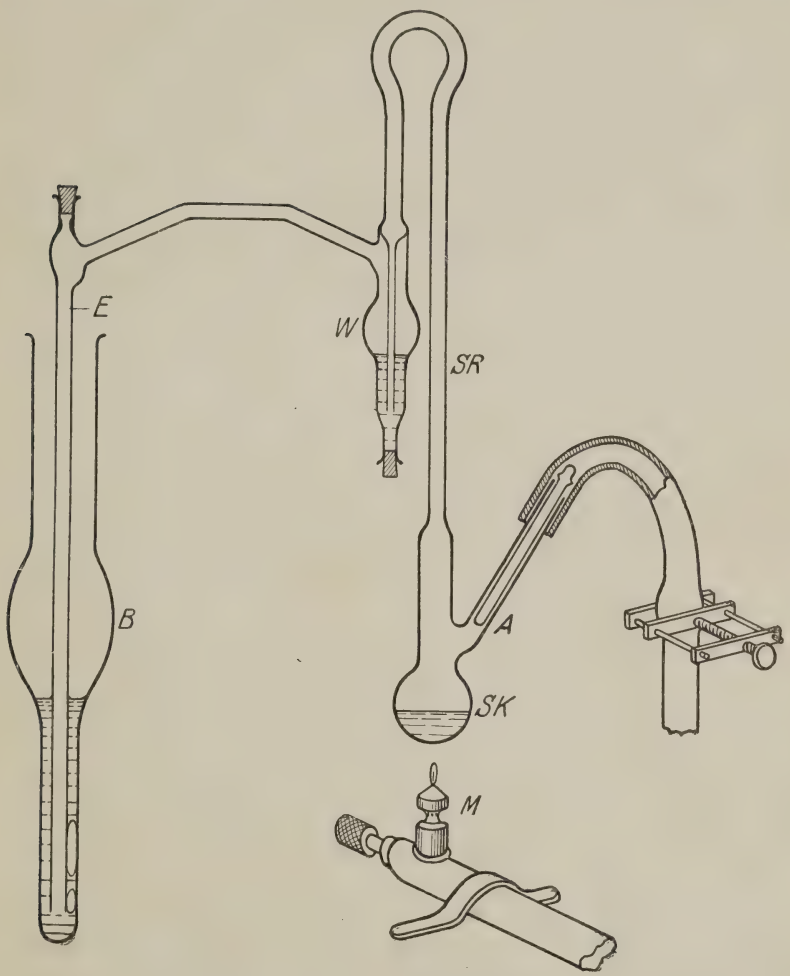

FIG. 33.—Apparatus for the micro-determination of methoxyl. (½ actual size.) *M*. Micro-burner. *SK*. Boiling flask with ascension tube. *SR*. and side tube *A* with glass rod and rubber tube with pinch-cock. *W*. Washer. *E*. Delivery tube. *B*. Expanded test tube.

fitting glass rod or, preferably, of a thin-walled sealed glass tube, as this is lighter.

In order to prevent this rod from slipping into the bulb, I originally attached a glass bead to the side tube. Latterly I found it preferable to furnish the glass rod with a small collar which

serves the purpose much better. If the side tube terminates at a somewhat oblique angle, any obstruction to the gas current is avoided. The ascension tube *SR* of the boiling flask is bent over at the top, and is sealed to the washer *W*, which contains an aqueous suspension of washed red phosphorus ; it is filled from below, to a quarter of its height, before each determination, by means of a narrow glass tube, whilst the apparatus is held in an inclined position. The washer is closed below by a very small cork. The washer is connected to the vertical gas delivery tube by a tube with an upward bend. It is well to bind to this bent portion a split perforated cork to enable the apparatus to be held in a clamp. If the perforation of the cork is somewhat wider than the outer diameter of the glass tube the apparatus is free to move slightly in all directions, and is, therefore, much less likely to be broken. The long, lower straight portion of the gas delivery tube *E* is open at both ends. The short upper part of the delivery tube, above the point where it joins the connecting tube, has one or two constrictions, produced by thickening the walls. Before using the apparatus a drop of water is introduced above, after which a cork is inserted into the upper opening. By means of this water seal I have found it possible to make a joint impermeable to the vapours of methyl-iodide and similar compounds without further complications.

As receiver, we used a test tube *B*, which is widened in the middle and stands on the bench top. This is charged with an alcoholic silver nitrate solution. It has been found by experience that methyl-iodide is quantitatively absorbed in the narrow lower portion of the test tube, if this latter has a diameter of 7 to 8 mm., and a length of 50 mm., as shown in the drawing. The silver nitrate solution in a second connected receiver never showed any turbidity.

Before commencing each determination the apparatus is emptied and rinsed with tap water, and, finally, with distilled water after removing both corks. It has been found absolutely necessary to dry the boiling flask before each determination. This is best done by attaching the connection from the pump to the side tube, and aspirating a rapid air current with gentle warming. In order that the precipitate of silver iodide may be removed quantitatively it is necessary that all glass surfaces which come into contact with this precipitate should be perfectly clean and fat-free. For this purpose, the washed widened test tube is filled with sulphuric-chromic acid mixture, into which the

delivery tube of the apparatus for the determination of methoxyl is placed. After a few minutes the delivery tube is rinsed without and within with distilled water. The washer is filled with the suspension of phosphorus by holding the apparatus in an almost horizontal position, and running in the suspension by means of a drawn-out glass tube. A few particles of phosphorus are sufficient, and excess should be avoided. The outside of the gas delivery tube, after being rinsed with water, is finally washed without and within with alcohol, a precaution which has been found to be very advantageous. A drop of distilled water is then introduced into the upper opening with a wash bottle or glass rod, and the cork then inserted in order to form the water seal already mentioned. The apparatus is then fixed in a clamp by means of the cork; the receiver is carefully rinsed with water and with alcohol in order to remove the water, and is then filled with alcoholic silver nitrate solution up to a point a little above the top of the narrow portion. By lowering the clamp the gas delivery tube is lowered to the bottom of the receiver, which is then placed in a beaker on the bench top. The ascending bubbles are flattened on account of the narrowness of the passage, and an ample surface contact between them and the alcoholic silver solution is thus produced, which ensures the completeness of the absorption.

The apparatus is now charged through the side tube with 1·5 c.c. of pure hydriodic acid for methoxyl determinations (sp. gr. = 1·70), and then with 2 drops of acetic anhydride, or a few crystals of phenol,[1] or sometimes with both ; both have proved very satisfactory. Finally the weighed material is added.

The side tube is closed by inserting the glass stopper, and is then connected with a Kipp carbon dioxide generator by rubber tubing which passes over the protruding end of the stopper and over the side tube. Into the other end of this tubing there have previously been inserted, with the help of a wire, a few pieces of cotton-wool or of soft string, and a screw pinch-cock is provided at this spot. By this means it is possible to adjust the gas current with extreme accuracy, and it is easily reduced to such a point that there are never more than two gas bubbles passing through the silver nitrate solution at any one time.

A very small non-luminous flame from a micro-burner is now placed at a distance of about 15 mm. below the boiling flask. The consequent warming produces an acceleration of the gas current, and it would be a mistake to alter the pinch-cock

[1] F. Weishut, *Monatshefte f. Chemie* (1912), **33**, p. 1165 ; (1913), **34**, p. 1549.

accordingly, as after boiling commences the ascending bubbles resume their original rate. After not more than 3 minutes the first indications of the formation of a precipitate at the lower end of the delivery tube are observed. The precipitate, which is at first coarsely flocculent, gradually becomes crystalline, and does not usually show any apparent increase after 8 or 10 minutes. In spite of this, it is better to allow the liquid to boil for 20 minutes, so that the last traces of methyl-iodide may pass over into the receiver.

The operation is concluded by removing the burner, raising the apparatus with the clamp so that the lower end of the delivery tube reaches into the empty wider portion of the receiver, removing the cork from the water seal, and rinsing the delivery tube with water, first outside and then from above with a sharp jet from the wash bottle. Should a few particles of silver iodide still adhere to the delivery tube, this is again rinsed with a few drops of alcohol, and then once more with water as already described. By alternately rinsing with alcohol and water, all adherent particles of precipitate are readily removed with the help of the surface tension effect, provided that the glass surface was originally clean and free from grease. Should a particle of any size be obstinately retained at any point it can be successfully removed by using the feather. The contents of the receiver are diluted by the washings, so that their final level is at about the middle of the widened portion. After adding 5 drops of concentrated nitric acid free from halogen, the receiver is placed in a gently-boiling water bath until the contents commence to boil. It should be expressly noted that it has been found quite unnecessary to evaporate the alcohol as recommended for macroanalysis, and that, after warming for one or two minutes, the silver halide double compound is completely decomposed. After cooling under the tap, or in a beaker filled with cold water, the separated silver iodide is aspirated into a filter tube in the manner already described and duly weighed.

Preparing the Material for the Methoxyl Determination

I originally weighed the material in glass capillaries, 1 mm. wide and 12 mm. long, which were first weighed when empty, and then after filling them by pushing them into the dried material, by which means 3 to 5 mgm. could be introduced. After thoroughly wiping the capillary the second weighing was so carried out that the filled end projected beyond the edge of the balance pan. In

order to avoid loss during further handling, a square piece of tin-foil, of 6 mm. edge, was placed on the note book, and the capillary placed horizontally on this, so that the filled end rested in the middle of the square. By folding and twisting the tin-foil any further loss could be avoided. The first experiments on these lines showed the advantage of the presence of such small amounts of tin in carrying out the determination of methoxyl. Hydriodic acid, which is otherwise inclined to bump, boils perfectly quietly under the influence of the separated stannous iodide. Therefore the introduction of fragments of porcelain or other similar material for preventing bumping is quite superfluous when the methoxyl determination is carried out in this form.

Since 1913, I no longer use glass capillaries for weighing the material, but have replaced them by tin-foil receptacles. A square piece of tin-foil, of about 16 mm. edge, is converted into a regular octagon by cutting off the corners and then shaped over the end of a glass rod, 5 mm. in diameter, with rounded edges, to form a cup, which is easily removed by turning it in the reverse direction round the rod, and is then weighed. It was found that the methoxyl values which were obtained were too low if these tin-foil cups were too large and heavy. This is comprehensible, as larger amounts of tin would appreciably decrease the concentration of the hydriodic acid. Since we have regularly avoided the use of tin-foil cups weighing more than 20 mgm., and have carefully dried the boiling flask before each determination, the methoxyl determinations have been uniformly successful.

The weighed cup is transferred from the balance to the note-book, filled with 3 to 5 mgm. of the substance to be analysed by means of a knife point, and is then rolled into a small parcel with three fingers of the right hand, weighed after a short interval, and then carried to the apparatus on a copper block in the desiccator.

Red Phosphorus

The commerical product is not suitable for the methoxyl determination without further treatment. It is purified by heating for half an hour on the boiling-water bath with strongly ammoniacal water, filtering on the pump, washing exhaustively with water, and finally with alcohol. It is then stored in wide-necked stoppered glass bottles under water. For each determination the supernatant water must be decanted and replaced by fresh water, with which the phosphorus sediment is shaken up. It should be specially noted that preparations of phosphorus, the

water of which has been changed daily for almost a year, always show a noticeable smell of phosphine on the following day.

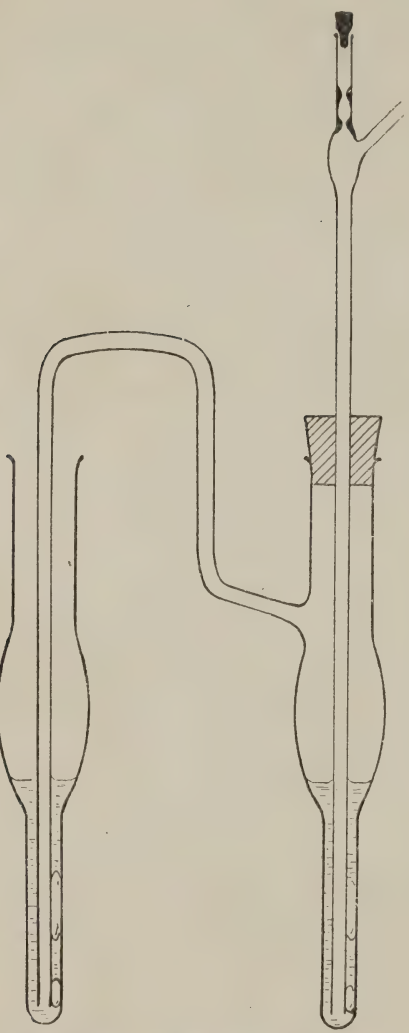

FIG. 34.—Receiver used for the determination of methoxyl- and methylimido-groups when using pyridine as absorbent. ($\frac{1}{2}$ actual size.)

Hydriodic Acid

As hydriodic acid, which has been most carefully prepared for the determination of methoxyl, of sp. gr. 1·7, can now be obtained from the firms of E. Merck, of Darmstadt, and Kahlbaum, of Berlin, it is not worth while to prepare and purify this reagent in the laboratory. All conditions which promote the decomposition of hydriodic acid and separation of iodide, such as exposure to light, should be carefully avoided. We found that a preparation, which, after standing for two years, had a very deep brown colour and was opaque, yielded hydriodic values which were too low, obviously because the concentration of the undecomposed hydriodic acid was lower than that corresponding to sp. gr. 1·7. In very exceptional cases it might be necessary to use hydriodic acid of sp. gr. 1·90.

Alcoholic Silver Nitrate Solution

20 gm. of silver nitrate are dissolved in 200 gm. of 95 per cent. alcohol, and the solution boiled for three to four hours on a water bath under reflux. During this process reduced silver separates in a very finely-divided state. After standing for one to two days the liquid is decanted into a large dark stock bottle.

Dr. Hans Lieb undertook the investigation of the modified microanalytical determination of methoxyl recommended by Kirpal and Bühn, of Prague.[1] He found that a quantity of pyridine equal to that of the silver nitrate solution ordinarily used was insufficient completely to absorb the methyl iodide which was formed. Therefore the first widened receiver was replaced by one otherwise similar, into which a delivery tube was sealed, which passed to the bottom of a second receiver of the usual pattern. The first receiver was closed by a tight cork, through which the gas delivery tube of the methoxyl apparatus was passed (Fig. 34).

When this arrangement was used the methyl iodide was completely absorbed. Both receivers were emptied into a flat-bottomed evaporating dish and quantitatively rinsed with alcohol. After evaporating on the water bath and dissolving the residue in water a minimal amount of potassium chromate is added, preferably by means of a glass hair capillary, and 0·001 N silver nitrate solution is added until the first traces of a weak brown tint appear. This colour change is easily observed by daylight after some practice, but is rather difficult to observe by artificial light. Although the results are extremely satisfactory, and, moreover, the pyridine method can be safely used for substances containing sulphur, the gravimetric method of determining methoxyl is to be preferred, as it can be carried out with artificial light and is quite as rapid as the titrimetric determination. The gravimetric method of microanalysis can be equally well applied to substances containing sulphur if the aqueous suspension in the washer of the apparatus is replaced by a suspension of red phosphorus in a 5 per cent. cadium sulphate solution.

Some Analytical Results of the Micro-Determination of Methoxyl

(*a*) *Gravimetric in the absence of Sulphur*

Vanillin :

3·750 mgm. : 5·78 mgm. AgI.	= 20·37 per cent. OCH_3
Theory . . .	= 20·40 per cent. OCH_3

Veratric acid, $C_9H_{19}O_4$:

3·210 mgm. : 8·24 AgI . .	= 33·92 per cent. OCH_3
Theory . . .	= 34·07 per cent. OCH_3

[1] *Monatshefte f. Chemie* (1915), **36**, p. 853.

(*b*) *Gravimetric in the presence of Sulphur*

5 per cent. cadium sulphate solution was used for absorbing hydrogen sulphide in the washer of the apparatus.

Vanillin (to which potassium sulphate was added) :

4·030 mgm. (with 3·71 mgm. K_2SO_4) : 6·23 mgm. AgI . = 20·43 per cent. OCH_3

Theory . . . = 20·40 per cent. OCH_3

(*c*) *Volumetric with and without Sulphur, by use of pyridine as an absorbent for Methyl iodide by the Kirpal method*

Vanillin, without addition :

5·750 mgm. : 3·73 c.c. N/100–$AgNO_3$ . . . . = 20·13 per cent. OCH_3

Theory . . . = 20·40 per cent. OCH_3

Vanillin (with addition of potassium sulphate) :

6·084 mgm. (with 2·167 mgm. K_2SO_4) : 3·94 c.c. N/100–$AgNO_3$ . . . . = 20·10 per cent. OCH_3

Theory . . . = 20·40 per cent. OCH_3

Regarding the historical development, criticism and literature relating to this subject the reader is referred to the exceedingly thorough article, " Über Methoxyl-und Methylimidbestimmung " (" On the Determination of Methoxyl and Methylimide ") by J. Herzig, of Vienna, in Abderhalden's " Handbuch der biologischen Arbeitsmethoden, 1921, pp. 509–534, and also in the Appendix to that work, pp. 917–919.

Maximilian Ripper and Franz Wohack described a volumetric microanalytical method of determining methoxyl in glycerine and alcohol, which is based on the process of A. Klemenc. Further particulars may be found in *Zeitschr. f. d. landwirtschaftliche Versuchswesen in Österreich* **19**, 372 ; **20**, 102 ; *Monatshefte f. Chemie,* **34**, 6, and in " Handbuch der biologischen Arbeitsmethoden 1921," I., 3/3, pp. 547–552.

XII. The Microanalytical Determination of Methyl Groups attached to Nitrogen

After the microanalytical determination of methoxyl had been developed the determination of methyl groups attached to nitrogen was investigated. An experiment on this subject was actually tried at Innsbruck in 1913, though with very moderate success, as the flask cracked after one or, at most, two determinations

by the method then employed, and the hydriodic acid could not yet be returned to the reaction flask with complete accuracy and convenience.

In the winter of 1915, I attacked the problem once more, in

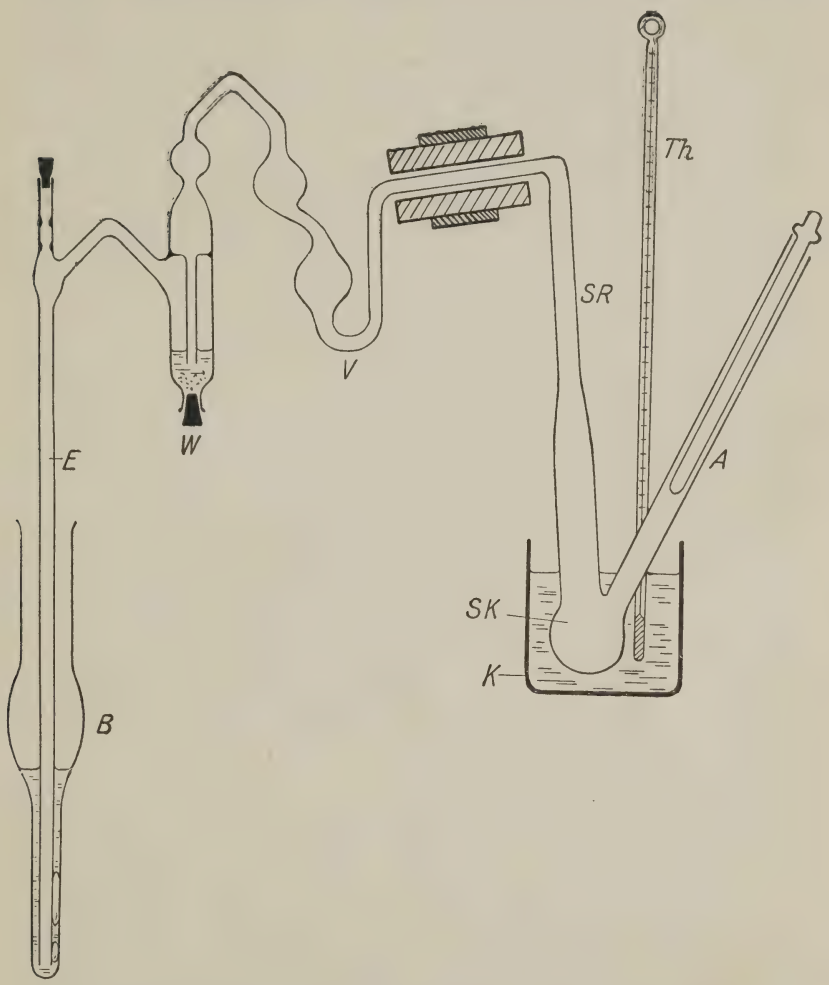

FIG. 35.—Apparatus for the determination of methylimide. *K.* Copper bath filled with molten vaseline, the temperature of which is measured by the thermometer *Th.* *SK.* Boiling flask. *SR.* Condenser tube. *V.* Receiver. *W.* Washer. *E.* Delivery tube. *B.* Widened test tube.

conjunction with Dr. Lieb. Many forms of apparatus were at first tried, but the following proved to be satisfactory (Fig. 35).

Suitable apparatus was devised, consisting of a small flask *SK*, of about 20 mm. diameter, provided with a condenser tube *SR*, at an angle slightly inclined to the vertical, 150 to 160 mm. long, and

of 6 to 7 mm. outer diameter. Shorter and wider tubes rendered it necessary to distil repeatedly, whereas with these dimensions, practically the whole of the nitrogen attached to methyl groups was driven off by the first distillation. The side tube *A*, for the introduction of the material and of the current of carbon dioxide, was made longer in this case than in the apparatus for the determination of methoxyl, being at least 100 to 140 mm. long. It is better to attach this side tube above the spherical portion of the flask rather than to the actual bulb, as the life of the apparatus is then considerably longer. The condenser tube *SR* is bent over at the top so that it is slightly inclined to the horizontal for a length of 60 mm., and then descends vertically downwards into the receiver *V*. The almost horizontal portion is surrounded by a split cork, as in the methoxyl apparatus, so that the whole apparatus may be supported by a clamp at this point.

The receiver *V* for the distilled hydriodic acid originally consisted of a single vertical, cylindrical vessel ; it has, however, been found advisable to replace this by two smaller bulbs connected by an oblique tube, in order completely to avoid spirting of the hydriodic acid. The washer *W*, which follows, differs from the washer of the methoxyl apparatus, being surmounted by two wider portions, which are so large that on sucking back the hydriodic acid before commencing a fresh distillation the whole suspension of phosphorus in the washer is retained by them.

The gas delivery tube *E* which follows the washer is exactly the same as in the apparatus for the determination of methoxyl, and the same form of widened test tube *B* is used for holding the alcoholic silver nitrate solution.

During our first experiments in Innsbruck we heated the apparatus in a bath of iron filings, similar to the sand bath used by Herzig and Meyer in the macroanalytical determination. In consequence of this manner of heating, the flask never survived more than two experiments. I am indebted to Professor Emich for his friendly suggestion that a bath of vaseline could be used for temperatures up to 400° C. We now always heat the flask in crude vaseline, contained in a vessel of stamped copper *K*, of 500 mm. diameter and 50 mm. high. The temperature can be accurately read on a thermometer *Th*, dipping into the liquid.

In order to carry out a determination the washer is charged with a suspension of phosphorus, exactly as in the determination of methoxyl, and closed with a suitable cork. The flask is then charged with two knife points full of ammonium iodide (that is

10 to 20 times as much as the amount of material to be analysed), then with about 1·5 c.c. of hydriodic acid (sp. gr. 1·7), and, finally, with the weighed material in a twisted tin-foil cup. The side tube is then closed with the small rod and connected with the Kipp carbon dioxide generator by means of the tubing. The gas delivery tube has already been cleaned, as previously described, in sulphuric-chromic acid, water and alcohol, provided with a water seal, and immersed in alcoholic silver nitrate solution contained in the widened test tube, to the bottom of which it reaches.

By heating the hydriodic acid so that it is only just at the boil, it is possible to determine the methoxyl only in substances containing that group ; this is effected by stopping the determination, rinsing out the delivery tube, and further treating the precipitate which has been obtained as already described in connection with that determination. If a freshly charged receiver is then used and the temperature gradually raised, then when it has attained a certain height, which differs with different substances, but is above 220°, and usually below 300° C., the methyl groups are split off in the form of methyl iodide. Although methoxyl groups are completely liberated within twenty minutes, the liberation of methyl groups attached to nitrogen usually requires an hour or rather more. The experiment is ended by extinguishing the flame, and allowing the vaseline bath to cool to 150° C., whilst the current of carbon dioxide is continuously maintained. The receiver is then lowered, the delivery tube rinsed in order to remove the last traces of precipitate, and the silver iodide determined in the contents of the receiver exactly as in a methoxyl determination. In the microanalytical determination of the methyl groups it is necessary to repeat the decomposition and distillation just as in the macroanalytical method. For that purpose a rubber tube is slipped over the inlet tube for the carbon dioxide, and the hydriodic which has collected in the receiver *V* is carefully sucked back into the flask. The bulb at the top end of the washer prevents phosphorus suspension from being carried over into the receiver, which is intended for hydriodic acid only. If the operations are then repeated, a second separation of the silver iodide double compound occurs in the receiver when the temperature of decomposition is attained ; in the case of some substances a third distillation is necessary in order that the reaction may be carried to completion, which is only the case when the quantity of silver iodide is below 0·5 per cent.

Dr. Lieb also tried absorption by pyridine when determining methyl groups attached to nitrogen. This is carried out exactly as described in the determination of methoxyl, but is of more importance in the present case, for, as is well known, pyridine absorbs methyl iodide alone easily and completely, whilst the other alkyl iodides are either not absorbed at all or absorbed incompletely. It is therefore possible, by means of the difference of the results obtained gravimetrically and volumetrically, to decide whether all the alkyl groups indicated by the gravimetric method are really methyl groups or not.

Some Analytical Examples of the Microanalytical Determination of the Methylimido-Group

(*a*) *Gravimetric*

Theobromine, $C_5H_2(CH_3)_2N_4O_2$

1. 3·780 mgm.

1st distillation . .	8·45 mgm. AgI =	14·30 per cent. CH_3
2nd distillation . .	0·74 mgm. AgI =	1·25 per cent. CH_3
	9·19 mgm. AgI =	15·55 per cent. CH_3
Theory for . .	2 CH_3 =	16·68 per cent. CH_3

2. 4·005 mgm. :

1st distillation . .	4·22 mgm. AgI =	6·74 per cent. CH_3
2nd distillation . .	3·64 mgm. AgI =	5·82 per cent. CH_3
3rd distillation . .	1·66 mgm. AgI =	2·62 per cent. CH_3
4th distillation . .	0·73 mgm. AgI =	1·19 per cent. CH_3
	10·25 mgm. AgI =	16·37 per cent. CH_3
Theory for . .	2 CH_3 =	16·68 per cent. CH_3

Atropine, $C_{17}H_{23}NO_3$

5·740 mgm. :

1st distillation .	4·16 mgm. AgI =	4·64 per cent. CH_3
2nd distillation . .	0·54 mgm. AgI =	0·60 per cent. CH_3
	4·70 mgm. AgI =	5·24 per cent. CH_3
Theory for . .	1 CH_3 =	5·20 per cent. CH_3

Cocaine Hydrochloride, HCl. $N(CH_3)C_4H_{10}\begin{matrix}O\cdot CO\cdot C_6H_5\\COOCH_3\end{matrix}$

4·633 mgm. . .	3·20 mgm. AgI =	4·42 per cent. CH_3 attached to oxygen
	3·13 mgm. AgI =	4·34 per cent. CH_3 attached to nitrogen
Theory for . .	1 CH_3 =	4·42 per cent.

(*b*) *Volumetric*

Theobromine $C_5H_2(CH_3)_2N_4O_2$
5·628 mgm.

1st distillation	5·76 c.c. N/100 $-AgNO_3$ =	15·37 per cent. CH_3
2nd distillation	0·64 c.c. N/100 $-AgNO_3$ =	1·17 per cent. CH_3
	6·40 c.c. N/100 $-AgNO_3$ =	17·08 per cent. CH_3
Theory for 2 CH_3	=	16·68 per cent. CH_3

Very considerable improvements in the determination of the methylimido-group are due to Siegfried Edlbacher, and were published in *Zeitschrift f. physiolog. Chemie*, **101**, 278–287, 1918. He uses an apparatus which only differs from that already described in so far as the decomposition flask is made of quartz, and is joined to the remainder of the apparatus by a ground joint and two spring clamps.[1] According to our experience it would be better if the barrel of the ground glass joint, and not the collar, were on the quartz flask, as the present arrangement tends to cause the quartz collar to crack at higher temperatures in consequence of the fact that the glass cone expands to a greater extent, and we found that this actually occurred. With the opposite arrangement, on the contrary, this is not to be feared, nor does the joint become loose, as this is prevented by the spring clamps. Edlbacher also furnishes the apparatus with two washers instead of one, and fills both of these with a 5 per cent. solution of cadmium sulphate. This suffices completely to retain both hydriodic acid and hydrogen sulphide. He finds that the latter body is present in considerable amount in commercial hydriodic acid. According to him the distillation of iodine is completely prevented by the large quantity of hydriodic acid which is used and distilled, so that a suspension of phosphorus appears to be unnecessary, although red phosphorus may well be added to the cadmium sulphate solution. The most important improvement devised by Edlbacher is the addition of 1 or 2 drops of a solution of gold chloride acidified with hydrochloric acid ; this catalyst accelerates the separation of alkyl groups to such an extent that all the alkyl is separated by distilling once with hydriodic acid of sp. gr. 1·7, at a temperature of 300° to 360° C. within 30 minutes. Future experiments will alone decide whether this catalytic acceleration of the reaction by means of gold chloride applies to substances of all classes. In other respects the method used by Edlbacher is identical with that already described.

[1] The apparatus for the microanalytical determination of the methylimido-group, with quartz flask, is supplied by Heraeus, of Hanau, and also by Haack, of Vienna.

XIII. Molecular Weight Determinations in Small Quantities of Organic Substances by Determination of the Rise of Boiling Point

The excellent Beckmann thermometers of the firm of Siebert and Kühn, of Cassel, represent such an improvement in the determination of the temperature of small quantities of liquid that I was induced, in 1912, to attempt molecular weight determinations in small quantities of material. If we consider the calculation of molecular weight from rise of boiling point we find that, by reducing the weight of the solvent, a proportional reduction of the material used is possible. The above-mentioned firm supplied me, not only with their excellent thermometers, but also with a correspondingly small boiling vessel with internal condenser, in which, when using small platinum tetrahedra, weighing about 3 gm., about 1·5 c.c. of liquid only was required in order that the liquid might completely cover the small mercury bulb of the thermometer. It was found impossible to maintain a constant boiling point in these experiments by the methods previously employed for boiling the liquid. This was only achieved by departing from the principle of heating in stagnant air, and so altering the conditions that air in uniform motion was warmed and passed round the boiling vessel; in this manner a steady temperature could be maintained for several minutes. The foundations were thus laid for the construction of an apparatus, by means of which a molecular weight determination could be carried out by one introduction of 7 to 10 mgm. of material into 1·5 c.c. of solvent.

During the first successful preliminary experiments, in which I had the advantage of the assistance of Dr. Hans Lieb, concentric glass cylinders and tubes were used in order to prevent convection of air, and to ensure that the moving air current traversed a definite path.

The apparatus which is now used by me, and which is shown in perspective in Fig. 36, and in section in Fig. 37, comprises a stand, on to which a rod can be clamped and vertically adjusted. This rod is provided with two clamps, of which the lower supports the boiling vessel, and the upper the Beckmann thermometer, which is immersed in the same. This arrangement enables one to introduce these two most important portions of the apparatus into the heating chamber and to remove them from the same without altering their relative positions.

The heating chamber is supported on a brass plate T; this is centrally perforated, and is held by a bracket affixed to the foot of the stand. A circular ebonite stand with three feet, also furnished with a central perforation, is screwed to this metal plate. On its upper surface there are three concentric grooves. These grooves serve the purpose of maintaining two cylinders, Cy_2, Cy_3, in a concentric position, the larger outer cylinder Cy_1 being supported by the metal plate already referred to. The arrangement of these three cylinders will be clear from the accompanying drawings. The outer cylinder is 140 mm. high and 84 mm. in diameter; the second, 120 mm. high and 45 mm. in diameter; whilst the third, innermost, cylinder consists of a shortened lamp cylinder, 110 mm. high, 36 mm. wide at the base and 26 mm. wide at the cylindrical portion.

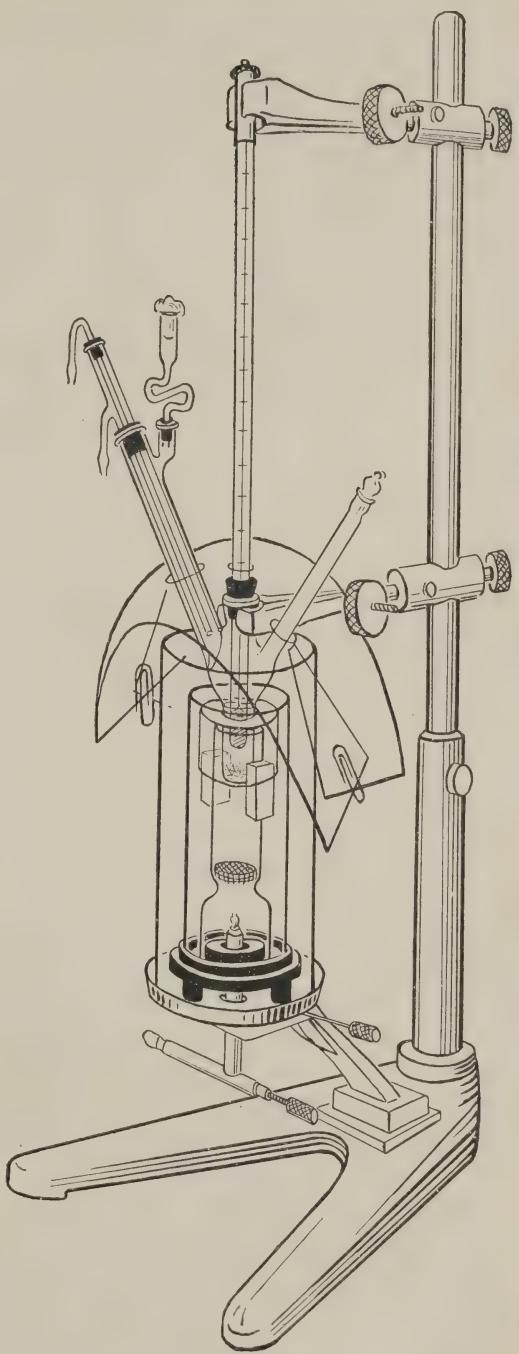

FIG. 36.—Apparatus for the determination of molecular weight of small amounts of material by the rise of boiling point. ($\frac{1}{4}$ actual size.)

A circular disc of copper or brass gauze, *Dr*, is supported by the constricted portion, and serves to warm uniformly the whole cross section of the ascending air current at this point. A fourth simple cylinder Cy_4, 26 mm. high and 36 mm. wide, surrounds the upper end of cylinder Cy_3, being held by three strips of asbestos board, which are interposed between these two cylinders. A mica plate is cemented to its upper opening with water-glass. A central perforation, of 16 mm. diameter, is cut into this plate with a suitable cork borer, and that part of the boiling vessel which is to be heated fits tightly into this perforation.

A micro-burner can be inserted through the central perforations of the metal plate and the ebonite stand from below. This is capable of delicate regulation and produces a non-luminous flame about 15 mm. below the wire gauze.

When assembling the apparatus the short fourth cylinder Cy_4 is first slipped over the lamp cylinder, and adjusted with the aid of the three asbestos strips, so that the mica plate is at a height of about 8 to 10 mm. above the lamp cylinder. After placing all the cylinders in position it is advisable to light the flame at once, in order to heat up the glass cylinders whilst the tablets are being prepared and weighed. To carry out a determination, the boiling vessel, which has previously been washed with sulphuric-chromic acid, water and alcohol, and dried in a current of warm air, is inserted into the lower clamp ; the platinum tetrahedra, which have been ignited immediately beforehand, are added and the Beckmann thermometer is inserted with the help of a well-fitting cork, so that the mercury bulb reaches almost to the tetrahedra, without touching them, its upper end being held by the upper clamp. The upper clamp is then loosened, the Beckmann thermometer removed, and 1·5 c.c. of the solvent run on to the platinum tetrahedra from a weighed pipette. The vessel is then closed at once by reinserting the thermometer, and the cooling water in the condenser is turned on. By adjusting the rod, to which both clamps are attached, the bottom of the boiling vessel, which has so far been held at a higher point to one side of the ascending current of hot air, is now brought over the central perforation of the mica disc, and then lowered until the boiling vessel is surrounded by the mica disc at the level of the upper surface of the liquid. On further lowering the boiling vessel, the fourth cylinder is forced downwards slightly over the third.

The path traversed by the hot air issuing from the apparatus is clearly indicated in Fig. 37, from which it is also clear how the

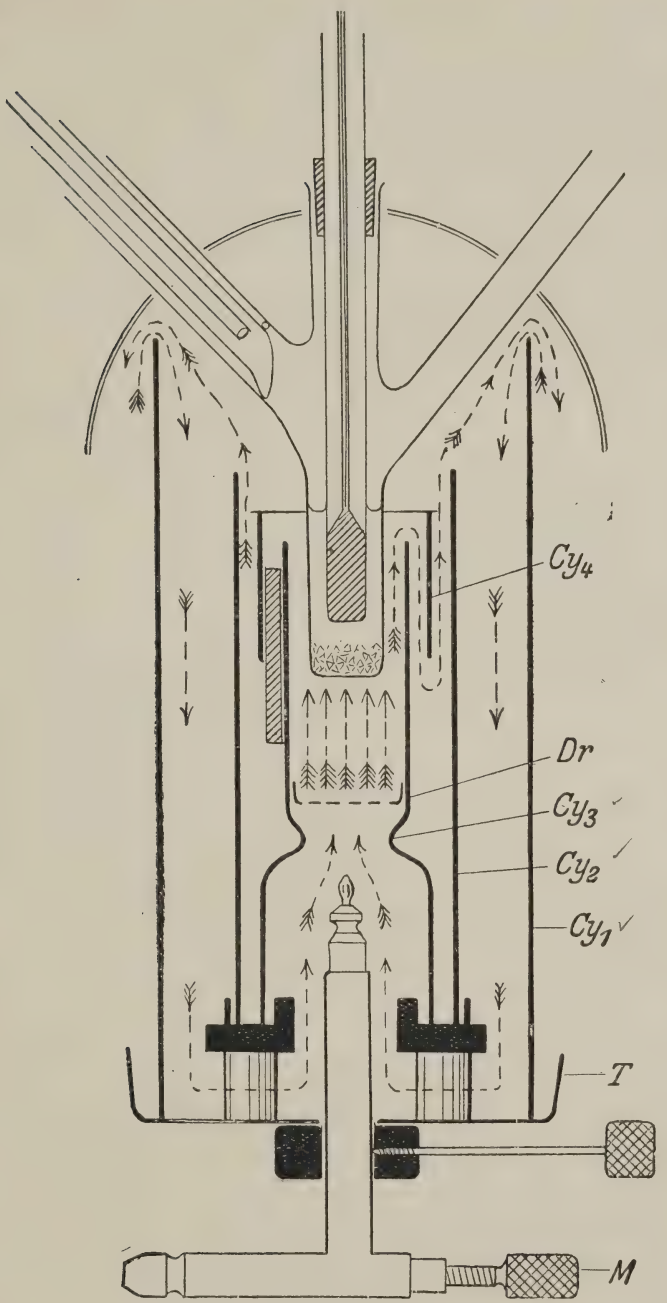

FIG. 37.—Apparatus for molecular weight determination in small amounts of material by the rise of boiling point. (Section.) ($\frac{2}{3}$ actual size.) *T*. Metal plate. *M*. Micro-burner. Cy_1, Cy_2, Cy_3, Cy_4. Four glass cylinders, with mica plate cemented to Cy_4. *Dr*. Wire gauze.

whole apparatus is fully protected from above by a plate of transparent celluloid. This consists of a rectangular sheet 200 mm. long and 130 mm. wide, in the central line of which three round holes are cut with a cork borer, at intervals of 35 mm., to fit the three upper extensions of the boiling vessel. In order to enable this celluloid cover to be placed over the apparatus it has three slits, which reach from its narrow edges to the two side holes, and from the middle of one of the longer edges to the central hole. After this cover has been placed in position the edges of the two ends are superposed, and held in position with ordinary letter clips as shown in the figure.

A number of conditions must be observed in order to ensure regular boiling. The flame must always be so regulated that the main cock leading to the micro-burner is turned on to the full extent, and the burner is then turned down to such a point that the micro-flame obviously becomes smaller. Secondly, it is advisable to introduce a pinchcock at a point in the rubber tubing, and preferably to introduce a piece of cotton-wool or string into the tubing beneath the pinchcock. The pinchcock is then closed to such an extent that the size of the micro-flame is further reduced. Finally, the fine screw regulator of the micro-burner *M* is adjusted in order to further reduce the flame by a barely perceptible amount. I also introduce into the circuit a small pressure regulator similar to that employed in the determination of carbon and hydrogen, with the one difference that the long central tube is omitted.

The internal condenser is very important for the maintenance of constant ebullition, for if the condensed solvent falls back drop by drop the temperature falls as each drop is returned. This occurrence is particularly disturbing in the case of solvents of high boiling point. If, therefore, the lower point of the internal condenser has much play within the apparatus its position will be altered each time the latter is touched, and, therefore, the continuous return flow of the condensed liquid may be interfered with. Any such interference with the flow of the returned liquid must be avoided by sealing glass beads to the point of the condenser or, still better, by the method which I have used latterly, of sealing a short length of platinum wire to its lower point.

The apparatus is so well protected from air currents that violent movements of a cloth in the immediate neighbourhood cause no change in the temperature registered by the thermometer. This is influenced, on the other hand, by illumination by direct sunlight, particularly if this is intermittent. The determination is,

therefore, always carried out by us in the absence of direct sunlight. An interval of fifteen minutes is usually required after the liquid has commenced to boil, until the mercury thread takes up a steady position as observed by the lens which is also used with the micro-azotometer. This is due to the fact that the glass of the cylinders requires this amount of time in order to become uniformly heated. Experience has shown that a constant temperature is more quickly attained, and more accurate values obtained, by boiling fairly vigorously. Before each reading the tendency of the mercury to stick is overcome by gentle tapping

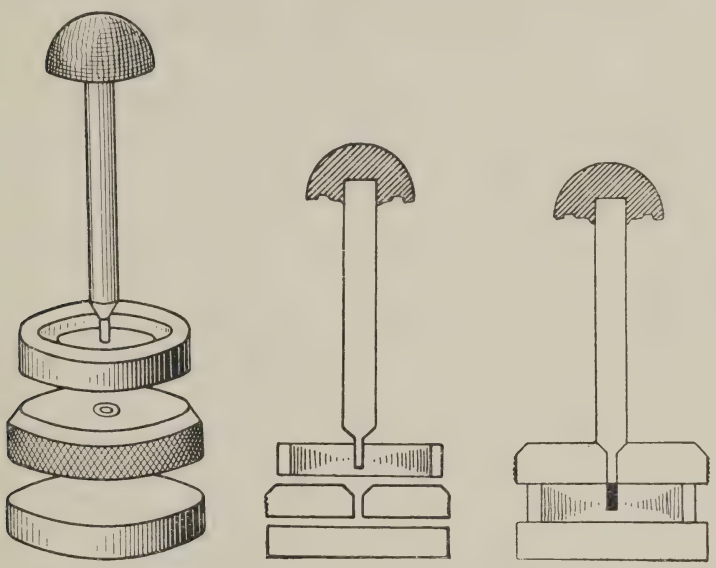

FIG. 38.—*a*. Tablet Press, side view. *b*. Section. *c*. Expulsion of finished tablet. ($\frac{1}{2}$ actual size.)

with a glass rod covered with a piece of rubber tubing, or by alternate tapping with two fingers. After the mercury column has remained steady within 0·002° C. during several minutes, the substance may be introduced in the form of weighed tablets.

It was necessary to construct a suitable tablet press (Fig. 38, *a*, *b*, *c*,) for the production of small tablets. Such a press was constructed to my specification by the University mechanic, F. X. Eigner, at Innsbruck. It consists of three portions. Firstly, a round base plate, of 45 mm. diameter, with a polished upper surface ; secondly, a steel plate of the same diameter and 9 mm. thick, with a central smooth-walled perforation of 2 mm. diameter, the top of which has a funnel-shaped expansion. If this

Fig. 39. — Charging tube. (Actual size.)

plate is placed on the base plate, with the funnel-shaped expansion upwards, its central perforation is closed completely by the base plate. On introducing the substance into the bore-hole through the funnel-shaped expansion, and pressing it home once by means of the piston of the compressor, which is furnished with a handle, a comparatively light pressure with the hand suffices to form a coherent tablet in the bore-hole. In order to remove this, a metal ring, of 8 mm. width, is placed between the base plate and the plate containing the tablet, and the latter is expelled by a sharp blow with the piston of the compressor, which causes it to be shot out into the interior of the ring on to the base plate. The base plate and ring are now supplied in one piece, so that the level surface is placed uppermost during compression and the opposite face when expelling the tablet.

We found by experience that it was advisable to produce tablets in this manner of a weight of 7 or, at most, 10 mgm. They are weighed with an accuracy of 0·01 mgm. only, in weighing tubes such as are used in the determination of nitrogen. The first tablet is introduced into the apparatus as soon as the boiling point has become constant. In order to prevent the tablet from hanging in the side tube it is advisable to place it after weighing in a small charging tube, of 3 to 4 mm. diameter and 15 mm. long, the closed end of which is provided with a slight pit, with a handle consisting of a glass thread, 150 mm. long and 1 mm. thick (Fig. 39).

This is introduced as far as possible into the side tube, and the tablet released by rotation. After solution has occurred, the boiling point rises gradually at first, then more rapidly, and finally acquires a constant value within 2 to 3 minutes at most. The second tablet is then introduced, so that the actual determination of the molecular weight of the material, introduced

in two portions, takes five to six minutes at most, if all the conditions are correctly observed.

As the recovery of the dissolved substance after such a determination offers no difficulties, it is usually possible, after recrystallisation, to dispose of 12 to 15 mgm. of material, in which all the elements may be determined. It is hardly necessary to insist on the scientific value of the possibility which now exists of determining not only the elementary composition, but also the molecular weight of minute quantities of valuable material.

Molecular Weight Determinations

I.—Ethyl Alcohol as Solvent

L = 1·175 gm.
K = 11·5

Naphthalene. *Mol. wt.* 128.

s_1 = 14·44 mgm.	s_2 = 28·42 mgm.
Δ_1 = 0·122° C.	Δ_2 = 0·211° C.
M_1 = 126	M_2 = 132

Diethylester of Bilianic Acid. *Mol. wt.* 506.

s_1 = 14·00 mgm.	s_2 = 22·31 mgm.
Δ_1 = 0·028° C.	Δ_2 = 0·044° C.
M_1 = 489	M_2 = 496

II.—Benzene as Solvent

L = 1·34 gm.
K = 26·7

Azobenzene. *Mol. wt.* 182.

s_1 = 9·67 mgm.	s_2 = 22·57 mgm.
Δ_1 = 0·102° C.	Δ_2 = 0·245° C.
M_1 = 189.	M_2 = 184

Methylbenzanthraquinone. *Mol. wt.* 272.

s_1 = 10·28 mgm.	s_2 = 17·71 mgm.
Δ_1 = 0·079° C.	Δ_2 = 0·128° C.
M_1 = 259	M_2 = 276

III.—Glacial Acetic Acid as Solvent

Anthraquinone. *Mol. wt.* 208.

s_1 = 11·68 mgm.	s_2 = 20·46 mgm.
Δ_1 = 0·107° C.	Δ_2 = 0·191° C.
M_1 = 205	M_2 = 201

Diphthaloyl-o-Naphtylenediamine. *Mol. wt.* 418.

$s_1 = 12{\cdot}43$ mgm.	$s_2 = 19{\cdot}83$ mgm.
$\Delta_1 = 0{\cdot}055°$ C.	$\Delta_2 = 0{\cdot}091°$ C.
$M_1 = 424$	$M_2 = 409$

IV.—Acetone as Solvent

$L = 1{\cdot}20$ gm.

$K = 16{\cdot}7$

Azobenzene. *Mol. wt.* 182.

$s_1 = 8{\cdot}30$ mgm.	$s_2 = 15{\cdot}50$ mgm.
$\Delta_1 = 0{\cdot}063°$ C.	$\Delta_2 = 0{\cdot}110°$ C.
$M_1 = 183$	$M_2 = 196$

Compound $C_{18}H_{16}N_2O$. *Mol. wt.* 276.

$s_1 = 13{\cdot}11$ mgm.	$s_2 = 23{\cdot}94$ mgm.
$\Delta_1 = 0{\cdot}066°$ C.	$\Delta_2 = 0{\cdot}113°$ C.
$M_1 = 276$	$M_2 = 295$

Finally, I wish to refer to the two methods of determination of molecular weight described quite recently by Rass. One of these is a modification of that originally suggested by Barger (*Ber.* **54**, 1979 (1921)), and in the other he uses camphor as a solvent and an ordinary apparatus for the determination of melting point for the measurement (*Ber.* **55**, 1051 (1921)). Though both are amazingly simple, their utility is limited by the small choice of available solvents, and often by slight solubility of the substance to be investigated ; for this reason my boiling point method still seems to be worth recording.

XIV. Note on the Purification of Small Amounts of Material

Methods of purification of small amounts of material of the order of a few centigrammes are generally known and practised, but in the case of amounts of a few milligrammes greater care and special methods are necessary in order to reduce the loss to a minimum.

When recrystallising such small amounts, for instance the residue from a molecular weight determination, we have found it advantageous to use the separated lower ends of ordinary test tubes of 14 mm. diameter and about 40 to 50 mm. long (Fig. 40). When rounding off the edge a small lip is easily provided. Solutions are prepared in such vessels above the small non-luminous flame of a micro-burner without any fear of loss of liquid by bumping in the narrow vessel if this is held at an oblique angle

and stirred with a small glass stirrer. This is prepared from a glass rod drawn out in the flame to a thickness of 1 mm. and about 20 mm. long. One end of this is thickened in the flame to an oblique hanging glass drop of 2 to 4 mm. diameter, whilst the other end remains cylindrical, and can be twisted during use between thumb and forefinger.

Filtration of hot solutions, which should preferably not be fully saturated, is best carried out by filtration through cotton-wool or asbestos-wool, which is introduced when dry into a so-called micro-funnel (Fig. 40) and lightly packed. Such micro-funnels are prepared from the upper ends of ordinary test tubes, which are drawn out in the flame, at a distance of about 40 mm. from the top, to a tube 1·5 to 2 mm. wide and 40 to 50 mm. long. The point at which the narrow portion of the tube would otherwise join the wide portion is slightly enlarged to a small bulb, of about 5 mm. diameter, to hold the filtering medium.

Fig. 40.—Micro-funnel and micro-beaker. (Actual size.)

It is best to filter the solution into one of the shortened test tubes already described, in which it is concentrated as desired over a micro-burner with constant stirring. It is often possible, by subsequently stirring the cooled solution, to cause substances to separate within a few minutes which normally crystallise very slowly.

Crystallised materials, weighing a few milligrammes only, can very conveniently be drained on the micro-vacuum funnel (Fig. 41) of Emil Schwinger, the construction of which is somewhat similar to that of Haushofer's paper filter. The lower portion consists of a thick-walled glass tube, 100 mm. long, of 10 mm. outer diameter and 2 mm. to 2·5 mm. bore. The upper end is ground flat at right angles to its length, and polished, whilst the lower end is cut away at an oblique angle in order to facilitate formation of drops. The upper portion is prepared from tubing of the same outer diameter, with a bore which is at most 1 mm. larger. It is expanded above to a funnel-shaped extension. It is 45 mm. long, of which the upper 35 mm. are expanded to an internal diameter of 10 mm.

The unexpanded lower end is also ground and polished at right angles to the axis, so that it fits fairly closely on to the upper end of the glass tube already mentioned.

These two portions are connected by a suitable piece of rubber tubing, half of which fits over either, after interposing between them a circular piece of hardened filter paper, which is cut out with a cork borer and fills the space between the two polished surfaces. To carry out a filtration of crystallised material the tubular lower portion of the micro-filter is fitted to an ordinary small filter flask by means of a perforated rubber stopper, or, if the mother liquor is also to be collected quantitatively, it is fixed into the central tubulature of a doubly tubulated glass bell, with a ground edge fitting on a glass plate. A small evaporating dish is placed inside the bell in order to collect the filtrate. The manner in which this apparatus is used is obvious. It may also be mentioned here that small wash-bottles with a fine jet, which are prepared from ordinary test tubes, have been found particularly satisfactory for transferring such small amounts of crystals to the filter and washing them. After all the liquid has been drained the rubber tubing is pushed completely over the lower tube, and the upper portion of the filter, which is now free, is transferred, together with the filter paper, to a watch glass, to which the crystals may be transferred by pressing them out by a fairly tightly-fitting, sharp-edged glass rod, cut off rectangularly, which is passed down the narrow bore of the filter.

FIG. 41.—Schwinger's vacuum filter. ($\frac{1}{2}$ actual size.)

For purifying small quantities of liquid by vacuum distillation, I have frequently used a small apparatus (Fig. 42) which may easily be prepared from a single test tube of ordinary size. For this purpose the closed end of the test tube is first drawn out to a narrow capillary, 200 to 300 mm. long. The remaining portion of the test tube is drawn out at two points to capillaries about 2 mm. wide and 20 to 25 cm. long, so that between these, and also between one of these and the first narrow capillary, sections of the test tube intervene of the original diameter and about 35 mm. long. The long structure thus obtained is then bent in a smoky flame to the shape indicated in the appended drawing, Fig. 42.

Into the mouth of the test tube a glass cock is fitted by means of

a perforated cork, through which it is connected with the pump. This glass cock is clamped horizontally to a stand, and carries the remainder of the apparatus, which requires no further support. Contrary to expectation, the apparatus so constructed is not easily breakable, in consequence of the high elasticity of the glass.

The liquid to be distilled is aspirated through the open part of the final capillary with the help of the pump, and enters the vertical distilling vessel. The end of the capillary is then drawn out in the flame to a hair capillary, so that minimal quantities of air are regularly admitted during the distillation, in order to avoid superheating. If this capillary is too wide, undistilled portions of the liquid may prime over into the receiver. Both the vertical distilling vessel and the horizontal receiver are placed in two small glass or porcelain beakers which are supported by stands. One of these contains a

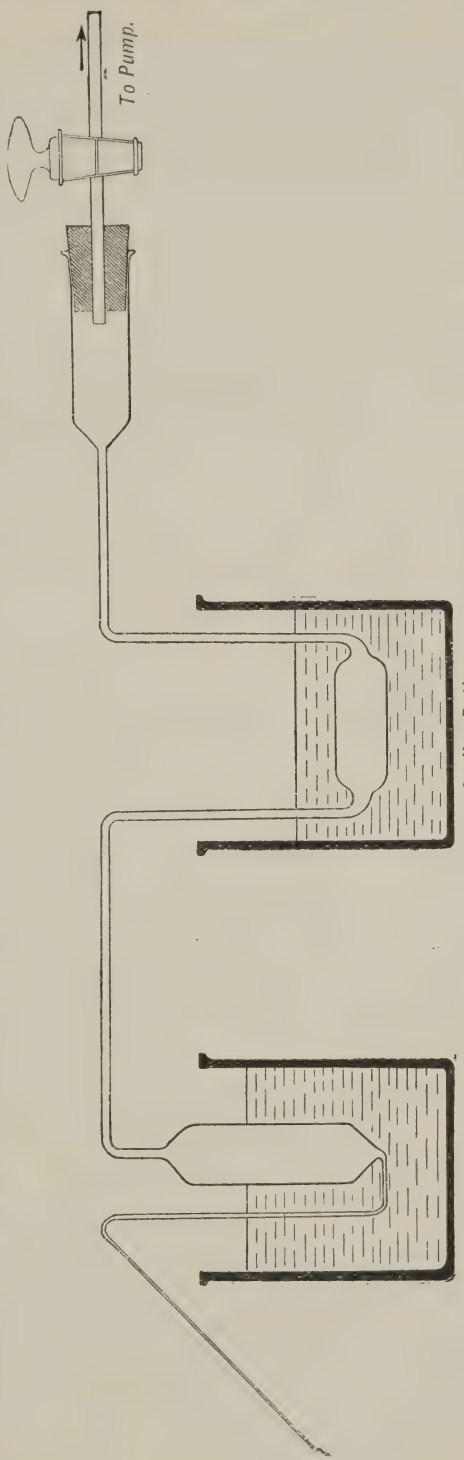

FIG. 42.—Micro-vacuum distillation. (Actual size.)

suitable heating liquid, and the other the condensing bath, to which ice is added if necessary. It is obvious that during the distillation the temperature of the heating bath only can be taken, and not that of the liquid undergoing distillation. After distillation is completed the liquid which has collected in the receiver is preserved from alteration until required by sealing the two capillary connections. I always recommend colleagues who wish to practise the combustion of liquids in my laboratory to purify nitrobenzene in this manner.

The regenerating block, which has already been described, has been found very suitable for the purification of small amounts of material by sublimation. If one wishes to sublime at ordinary pressure, all that is necessary is to place the material to be purified in a glass tube closed at one end, 200 mm. long and of 7 mm. external diameter, the closed end of which is introduced into the regenerating block. The temperature can be so exactly controlled that this operation can be carried out with great caution and without exceeding the temperature which is necessary. If the sublimation requires higher temperatures than those which can be obtained with the micro-burner supplied with the regenerating block, this can easily be replaced by an obliquely placed Bunsen burner. If it is necessary to sublime in a current of inert gas a longer tube is used, with a capillary constriction for the first third of its length, followed by a layer of asbestos, and, finally, by the substance which is to be purified. The necessary gas generator is connected to the shorter end of the tube which protrudes from the regenerating block. When subliming in vacuo the closed tube already described is naturally used, the open end being connected with the pump by rubber tubing. After subliming, the purified material is, in all cases, recovered by cutting off that portion of the tube in which the sublimate has collected, and then removing it by pushing it out with a sharp-edged glass rod on to a watch glass.

XV. Calculation of the Results of Microanalyses

This is always carried out logarithmetically, using exact atomic weights and F. W. Küster's logarithmic tables for chemists. I wish to call particular attention to the directions which Küster appends to Table 6, in the fourteenth edition of his book, pages 54 to 58, and to recommend that the trouble should never be spared of calculating the percentage of all the elements in any given substance from the formula, even although one or two elements

only are in question. Thus the percentage of oxygen should always be calculated. The accuracy of the calculations can then be checked, by ascertaining that the sum of the percentages amounts to 100. The necessary factors for the calculation of the analyses, and their logarithmic mantissæ, will now be given in the form recommended by Küster. For the convenience of the microanalyst we have also appended factors to be used in the volumetric determination of sulphur with the use of N/70 solutions, and also figures to be used in the determination of molecular weight by rise of boiling point, using 1·50 c.c. of the more usual solvents.

As factors for the determination of methoxyl-, ethoxyl- methyl- and carboxyl-groups are unfortunately missing in Küster's logarithmic tables, these are also given here, as they are frequently used in calculating formulæ.

Required	Found	Factor	Log
C	CO_2	0·2727	43 573
H	H_2O	0·1119	04 884
Cl	AgCl	0·2474	39 337
Br	AgBr	0·4256	62 896
I	AgI	0·5405	73 283
S	$BaSO_4$	0·1374	13 792
K	K_2SO_4	0·4487	65 198
Na	Na_2SO_4	0·3238	51 026
Mg	MgO	0·6032	78 044
Ca	$CaSO_4$	0·2943	46 883
Ba	$BaSO_4$	0·5885	76 971
Co	$CoSO_4$	0·3804	58 019
Cr	Cr_2O_3	0·6842	83 519
Al	Al_2O_3	0·5303	72 455
Fe	Fe_2O_3	0·6994	84 473
Mn	$MnSO_4$	0·3638	56 083
Cu	CuO	0·7989	90 250
Pb	$PbSO_4$	0·6831	83 449
Pb	PbO	0·9283	96 768
Pb	PbO_2	0·8662	93 760
Sn	SnO_2	0·7877	89 634
$O{\cdot}CH_3$	AgI	0·13213	12 100
$O{\cdot}C_2H_5$	AgI	0·19182	28 290
CH_3	AgI	0·06399	80 609

1 c.c. of N/70 solution corresponds to 0·2291 mgm. S.; log= 35 997.

1 c.c. of N/100 solution corresponds to 0·16035 mgm. S.; log = 20 507.

1 c.c. of N/100 silver nitrate solution corresponds to

0·3102 mgm. $O{\cdot}CH_3$; log = 49 170

0·1502 mgm. CH_3; log = 17 697

Multiples of

	$O.CH_3$	*log.*		$O.C_2H_5$	*log.*
1.	31·02 (4) ..	49 170	1.	45·04 ..	65 360
2.	62·05 ..	79 273	2.	90·08 ..	95 463
3.	93·07 ..	96 881	3.	135·12 ..	13 072
4.	124·10 ..	09 376	4.	180·16 ..	25 568
5.	155·12 ..	19 167	5.	225·20 ..	35 257
6.	186·14 ..	26 985	6.	270·24 ..	43 175
	CH_3	*log.*		*COOH*	*log*
1.	15·02 (4) ..	17 679	1.	45·008 ..	65 329
2.	30·05 ..	47 781	2.	90·016 ..	95 431
3.	45·07 ..	65 391	3.	135·024 ..	13 039
4.	60·10 ..	77 884	4.	180·032 ..	25 534
5.	75·12 ..	87 576	5.	225·040 ..	35 226
6.	90·14 ..	95 494	6.	270·048 ..	45 144

Calculations of Molecular Weight

$$M = K\frac{100 \cdot s}{L \cdot \Delta_t} = 100 \cdot \frac{Ks.}{L\Delta_t}$$

M = Required molecular weight.
K = Boiling point constant.
s = Weight of material.
L = Weight of solvent.
Δ_t = Observed rise of boiling point.

Ethyl Ether (b.p. 35°; $d_{15°} = 0{\cdot}720$).

v = 1·5 c.c. $\quad \frac{K}{L} = 19{\cdot}54$

L = 1·080 gm. $\quad \log \frac{K}{L} = 29\ 086$

K = 21·1

Acetone (b.p. 56·3°; $d_{15°} = 0{\cdot}797$).

v = 1·50 c.c. $\quad \frac{K}{L} = 14{\cdot}30$

L = 1·20 gm. $\quad \log \frac{K}{L} = 15\ 527$

K = 17·1

Chloroform (b.p. 61° ; $d_{15°} = 1{\cdot}50$).

$v = 1{\cdot}5$ c.c. $\qquad \frac{K}{L} = 16{\cdot}27$

$L = 2{\cdot}25$ gm. $\qquad \log \frac{K}{L} = 21\ 130$

$K = 36{\cdot}6$

Ethyl Alcohol (b.p. 78° ; $d_{15°} = 0{\cdot}793$).

$v = 1{\cdot}50$ c.c. $\qquad \frac{K}{L} = 9{\cdot}664$

$L = 1{\cdot}190$ gm. $\qquad \log \frac{K}{L} = 98\ 515$

$K = 11{\cdot}5$

Benzene (b.p. 80° ; $d_{15°} = 0{\cdot}885$).

$v = 1{\cdot}50$ cc. $\qquad \frac{K}{L} = 20{\cdot}11$

$L = 1{\cdot}328$ gm. $\qquad \log \frac{K}{L} = 30\ 331$

$K = 26{\cdot}7$

Water (b.p. 100° ; $d_{15°} = 0{\cdot}99913$).

$v = 1{\cdot}5$ c.c. $\qquad \frac{K}{L} = 3{\cdot}469$

$L = 1{\cdot}499$ gm. $\qquad \log \frac{K}{L} = 54\ 026$

$K = 5{\cdot}2$

Glacial Acetic Acid (b.p. 118° ; $d_{15°} = 1{\cdot}063$).

$v = 1{\cdot}50$ c.c. $\qquad \frac{K}{L} = 18{\cdot}77$

$L = 1{\cdot}598$ gm. $\qquad \log \frac{K}{L} = 27\ 354$

$K = 30{\cdot}0$

XVI. List of Some Researches carried out with the Help of the Microanalytical Methods described in this Work

Monatshafte fur Chemie

Vol. 33 (1912) :

R. Weitzenböck und H. Lieb. Eine Synthese des Chrysens, p. 561. C–H–.

G. Goldschmiedt. Über das Ratanhin, p. 1381. C–H– ; N– ; –O.CH_3.

VOL. 34 (1913):

J. Pollak. Über das Dithiobrenzcatechin, p. 915. C–H–; S–.

K. Brunner. Über Chinoncarbonsäureester, p. 919. C–H–; $-O.CH_3$.

E. Zerner und v. Löti. Zur Kenntnis des Euxanthons, p. 988. C–H–.

VOL. 35 (1914):

F. v. Hemmelmayr. Über einige Derivate von Di- und Trioxy-benzoesäuren, p. 1. C–H–; $-O.CH_3$.

I. Herzig und R. Stanger. Zur Kenntnis der Euxanthinsäure, p. 57. C–H–.

E. Philippi. Lineares Dinaphtanthracen, p. 375. C–H–.

G. Goldschmiedt und O. v. Fränkel. Uber γ-*p*-oxyphenyl propylamin, p. 383. C–H–; $O.CH_3$; Cl–; I–; Pt.

I. Herzig und P. Faltis. Zur Kenntnis des Bixins, p. 1006. C–H–.

E. Zerner und R. Waltuch. Zur Kenntnis der Pentosurie. p. 1025. N–; C–H–.

I. Pollak. Über Sulfurierung der Thiophenoläther, p. 1445. C–H–; N–; S– (gravimetric); S– (volumetric).

I. Pollak und A. Wienerberger. Über substituierte meta-Dimerkaptobenzole, p. 1467. C–H–; S–; Cl–.

VOL. 36 (1915):

E. Philippi und E. Spenner. Über den Verlauf der Einwirkung von Ammoniak und Harnstoff auf Ester ungesättigter Säuren, p. 97. C–H–; N–.

A. Eckert und K. Steiner. Chlorierungen cyklischer Ketone mit Antimonpentachlorid, p. 175. C–H–; Cl–.

A. Eckert und K. Steiner. Versuche über Perhalogenisierung des Anthrachinons, p. 269. Cl–; Br–.

K. Brunner. Eine neue Darstellungsweise von Triazolen, p. 509. C–H–; N–; Ag–.

VOL. 37 (1916):

H. Wolchowe. Triazole aus Dibenzamid, beziehungsweise Diparatoluylamid und Hydrazinsalzen, p. 473. C–H–; N–.

Zeitschrift für physiologische Chemie

VOL. 77 (1912):

E. Abderhalden. Bildung von Homogentisinsäure nach Aufnahme grosser Mengen von *l*-Tyrosin per os. p. 457. C–H–; H_2O–; Pn–; N–.

VOL. 83 (1913) :

H. Escher. Über den Farbstoff des corpus luteum, p. 205. C–H–.

VOL. 86 (1913) :

E. Abderhalden und Mitarbeiter, *d.l.* Aminocapronsäure, p. 454. C–H– ; N–.

VOL. 87 (1913) :

Hans Einbeck. Über das Vorkommen von Bernsteinsäure im Fleischextrakt frischen Fleisches, p. 145. C–H–.

R. Willstätter und M. Fischer. Untersuchungen über den Blutfarbstoff, I., p. 486. C–H– ; N–.

VOL. 88 (1913) :

A. Kossel und S. Edlbacher. Über einige Spaltungsprodukte des Thynnins und Percins, p. 186. C–H– ; N–.

VOL. 90 (1914) :

H. Einbeck. Über das Vorkommen von Fumarsäure im frischen Fleisch, p. 301. C–H–.

A. Windaus und A. Ullrich. Über die Einwirkung von Kupferhydroxydammoniak auf Traubenzucker, p. 366. N–.

VOL. 92 (1914) :

A. Windaus und A. Ullrich. Über die Einwirkung von Zinkhydroxydammoniak auf Rhamnose, p. 276. C–H– ; N–.

VOL. 93 (1914–1915) :

Karl Th. Mörner. Eine wohl charakterisierte organische Schwefelverbindung, erhalten aus Proteinstoffen bei Behandlung derselben mit Salpetersäure, p. 188. C–H–.

A. Kossel und S. Edlbacher. Einige Bemerkungen über das Histidin, p. 396. C–H– ; N–.

VOL. 94 (1915) :

A. Kossel und S. Edlbacher. Beiträge zur chemischen Kenntnis der Echinodermen, p. 264. C–H– ; N– ; mol. wt. determinations.

VOL. 95 (1915) :

H. Fischer. Über das Urinporphyrin, I., p. 50. C–H– ; N– ; $-O.CH_3$; Cu– ; Fe– ; Cl–.

P. Brigl. Synthetische Beiträge zur Kenntnis der Cerebronsäure, p. 190. C–H– ; N–.

Karl Th. Mörner. Über aus Proteinstoffen bei tiefgreifender Spaltung mit Salpetersäure erhaltene Verbindungen, p. 286. C–H– ; N–.

Vol. 96 (1915) :

H. Fischer. Über das Kotporhyrin, p. 148. C–H– ; N– ; –O.CH_3 ; Cu– ; Fe.

H. Fischer. Zur Kenntnis des Phylloerythrins, p. 292. C–H– ; N–.

Vol. 97 (1916) :

H. Wieland und H. Sorge. Untersuchungen über die Gallensäuren, II., p. 23. C–H–.

H. Fischer. Beobachtungen am frischen Harn und Kot von Porphyrinpatienten, p. 148. N– ; Zn–.

Liebigs Annalen der Chemie

Vol. 394 (1912) :

R. Scholl und Ch. Seer. Abspaltung aromatisch gebundenen Wasserstoffs und Verknüpfung aromatischer Kerne durch Aluminiumchlorid, p. 111. C–H–.

Vol. 398 (1913) :

Ch. Seer und R. Scholl. Zur Synthese des Violanthrons, p. 82. C–H– ; N–.

Vol. 399 (1913) :

O. Dimroth. Über die Karminsäure, p. 35. C–H–.

Vol. 400 (1913) :

R. Willstätter und Mitarbeiter. Über den Abbau der beiden Chlorophyllkomponenten durch Alkalien, p. 147. C–H– ; N–.

R. Willstätter und M. Fischer. Die Stammsubstanzen der Phyllin und Porphyrine, p. 182. C–H– ; N–.

Vol. 401 (1913) :

Fr. Straus. Zur Kenntnis des Physostigmins, I., p. 350. (Footnote, p. 371.) C–H– ; N– ; Cl– ; Au ; = N.CH_3.

Vol. 406 (1914) :

Fr. Straus. Über Physostigmin, II., p. 332. C–H– ; N–CH_3 ; N– ; Au.

Vol. 407 (1914) :

W. Steinkopf. Studien in der Thiophenreihe, VI., p. 94. S–.

Vol. 409 (1915) :

G. K. Almström. Über einige Pyrrolabkömmlinge, p. 291. C–H– ; N–.

Vol. 411 (1916) :

P. Pfeiffer. Lichtchemische Synthese von Indolderivaten, p. 72. C–H– ; N–.

VOL. 411 (1916) :

O. Dimroth und R. Fick. Über den Farbstoff der Kermes, p. 315. C–H–.

O. Dimroth und G. Schultze. Abbau von Oxyanthrachinonen zu Derivaten des Naphthochinons, p. 339. C–H– ; Br–.

Berichte der deutschen chemischen Gesellschaft

VOL. 45 (1912) :

H. Leuchs und E. Gieseler. Über Spirane, II., p. 214. C–H–.

VOL. 46 (1913) :

G. Goldschmiedt und E. Zerner. Über die Einwirkung von p-Bromphenylhydrazin auf Glucuron, p. 115. N–.

R. Weitzenböck und Chr. Seer. Zur Kenntnis des Perylens and seiner Derivate, p. 1994. C–H–.

VOL. 47 (1914) :

A. Windaus und C. Resau. Oxydationsversuche mit Cholesten, p. 1229. C–H–.

A. Windaus und L. Hermanns. Untersuchungen über Emetin, I., p. 1470. C–H– ; N–.

H. Wieland und R. Wishart. Die Synthese des natürlichen Inosits, p. 2082. C–H–.

H. Fischer. Über Mesobilirubin, p. 2330. C–H– ; N–.

VOL. 48 (1915) :

A. Windaus und C. Resau. Cholesterin, XX. Über die Oxydation des Cholesterylacetats mit Chromsäure, p. 851. C–H– ; Br–.

A. Windaus und Cl. Uibrig. Cholesterin, XXI., Über Koprosterin, p. 857. C–H–.

A. Windaus und L. Hermanns. Über Cymarin, p. 979. (See footnote, p. 981.) C–H– ; $O.CH_3$.

A. Windaus und L. Hermanns. Über die Verwandtschaft des Cymarins mit anderen Herzgiften des Planzenreiches, p. 991. C–H– ; $-O.CH_3$.

Th. Westphalen. Cholesterin, XXII. Über die Einwirkung von Benzopersäure auf Cholesterin, p. 1064. C–H–.

VOL. 49 (1916) :

E. Abderhalden und A. Fodor. Synthese von hochmolekularen Polypeptidenaus Glykokoll und *l*-Leucin, p. 561. C–H– ; Br–.

Vol. 49 (1916):

F. Ullmann and H. Bincer. Über die *l*-Chloranthrachinon-2-carbonsäure, p. 732. C–H–; N–; S–; Cl + Br.

A. Windaus. Cholesterin, XXIV. Überführung des Cholesterins in Koprosterin, p. 1721. C–H–.

Biochemische Zeitschrift

Vol. 54 (1913):

A. Baumann. Über den Stickstoffhaltigen Bestandteil des Kephalins, p. 30. C–H–; N–; Au–.

Vol. 63 (1914):

I. Hebting, Abbau der Chondroitinschwefelsäure über krystallinische Produkte, p. 335, C–H–; N–; Cl–.

Zeitschrift fur Biologie

Vol. 65 (1914):

H. Fischer. Zur Kenntnis der Gallenfarbstoffe, VI., p. 163. C–H–; N–.

Casimir Funk. Die Vitamine (Verlag Bergmann, Wiesbaden, 1914). C–H–; N–.

The Journal of Pharmacology and Experimental Therapeutics.

Vol. V., No. 6. July, 1914.

John Abel, L. G. Rowntree and B. B. Turner. On the Removal of Diffusible Substances from the Circulating Blood of Living Animals by Dialysis, p. 611. C–H–; N–.

A. v. Hoschek. Veränderungen des α-Methylindols im Lichte und einige Indolkondensationen. (Inaugural dissertation, Zürich University, 1915.) C–H–; N–, Molecular wt. determinations.

This list has not been extended beyond the year 1916 on account of the large quantity of material in which microanalysis of organic substances was used.

XVII. Final Remarks

In writing the present book I have endeavoured to describe all the conditions and manipulations which are necessary for the successful practice of microanalysis. I have therefore entered into an amount of detail which may in some instances appear unnecessarily extensive, so that the reader may be informed of all those apparently obvious minor precautions which may appear

hardly worth mentioning, either to the student or to private investigators.

The question as to whether my descriptions suffice in order to enable a gifted and able young chemist to carry out the methods with success without personal tuition, has been frequently answered. It has been found that this is always achieved under certain conditions, more particularly those of inborn "chemical cleanliness." I have, however, considered it necessary, in the interest of organic microanalysis, that as large a circle as possible from many chemical laboratories should be instructed personally by ourselves. I shall be glad to follow out this plan by carrying out microanalytical holiday courses in my institute in the near future, within the available limits of time and accommodation, just as in previous years, and extend a friendly welcome to all those who wish for instruction.

The course which has been carried out hitherto in such cases, is arranged in order of difficulty, more or less as follows :

After the beginner has become accustomed to the balance and its use, he first carries out the micro-Dumas method. After this has been practised on azobenzene, acetanilide, trichlorodinitrobenzene, nitrosodimethylaniline and nitrobenzene, he is instructed, on about the fourth day, on the details of the determination of carbon and hydrogen. He is usually asked to practise these determinations on the following materials : Quinizarine, cholesterine, perylene, anthraquinone, azobenzene, pyramidone, nitrodimethylaniline, methylcarbazole, o-iodocinnamic amide, *p*-chlornitrobenzene, *p*-bromnitrobenzene, trichlordinitrobenzene, sulphonal, barium bromo-benzene sulphonate, potassium *m*-benzene di-sulphonate and the liquids, nitrobenzene and ethyl alcohol.

This work requires, on an average, about eight full days. The most serious difficulties have then been overcome, and it is best to practise the micro-Kjeldahl or methoxyl determinations as a sort of recreation. During the latter determination the beginner practises the transference and weighing of silver halide precipitates. These determinations are naturally followed by instruction in the determination of halogen and sulphur. A further eight days at least are passed in this way.

Finally, according to inclination, the student proceeds to the determination of ash, molecular weight, copper by electrolytic methods, phosphorus or the methylimido-group.

Before the conclusion of this portion of the work I always recommend a further determination of nitrogen and of carbon

and hydrogen, so that all the details may be fully absorbed. A complete course of microanalytical work on this plan therefore usually lasts for three, or even four, weeks.

In working out my methods I have been supported during the course of many years by six firms whose help I must acknowledge here. I also mention them as sources of supply of the various apparatus necessary in microanalysis. They are :

1. W. Kuhlmann, manufacturer of precision balances, Steilshoperstrasse 103, Hamburg, supplies the microanalytical balance and weights, also counter-poise flasks and shot. Various occurrences have induced Mr. Kuhlmann to supply his apparatus to chemists only, and to avoid all agents.

2. Franz X. Eigner, at one time university mechanic at Innsbruck, supplied me with all metallic apparatus. For some years he has ceased to supply these, as he has taken up another occupation.

3. Wagner and Munz, Karlsstrasse 42, Munich, were of much assistance in the construction of my precision micro-azotometer. They also supply the pressure regulator. All supplies necessary for microanalysis, including metallic articles, may be obtained through them.

4. Paul Haack, Garelligasse 4, Vienna IX, constructs the distillation apparatus for carrying out the micro-Keldahl determination, and excellent absorption tubes for the determination of carbon and hydrogen, with carefully ground glass joints. He also supplies apparatus for the determination of methoxyl- and methylimido-groups. In addition to all glass apparatus described in this book, he also supplies the necessary reagents, platinum boats, platinum crucible, etc.

5. Dr. Siebert und Kühn, Cassel, supply the small Beckmann thermometers, also the small boiling vessel and platinum tetrahedra.

6. Anton Orthofer, mechanic to the Institute of Medicine of the University of Graz, has supplied metallic apparatus for microanalysis since the end of the war. On account of the scarcity of metals he has largely replaced copper and brass by aluminium, iron or steel, in most of the apparatus. Although the initial difficulties thereby incurred were considerable, such articles of excellent quality may be ordered from him, and are only sent out after careful testing.

INDEX

A

Abderhalden, 1, 15
Absorption apparatus, 35–43, 53, 55
  tubes, 39–42, 53, 56, 58, 65, 71, 72
Acclimatisation, 12
Acetone, 130
Ackermann, Prof. von, 5
Air, 17–18, 20, 65
Alcohol, 130, 149
Aluminium counterpoise, 14
  mortar, 32
Ammonia, 5, 82
Ammonium nitrate, 64, 130
  phosphate, 128
Arsenic, 5, 83, 133–135
Asbestos, Gooch crucible, 24, 108
  lead peroxide, 5, 26, 32, 51
  paper, 27, 34, 107
Azotometer, 76, 77, 89, 90, 92
  micro, 72, 73, 74, 85–87, 169

B

Balance, Kuhlmann's, 7–15
  microchemical, 7–15
Bang, Ivor, 101
Barium, 135
  chloride, 119
  sulphate, 119, 120, 121, 122
Benedetti-Pichler, Dr., 5
Bibliography, 6
Birkenbach, Dr., 29
Blank tests, 51–53
Blumer, Ferdinand, 42
Bregant, Dr., 3
Bromine, 114
Brunner, Carl, 3, 4, 40, 73
Bubble counter, 21, 22, 50, 52, 66, 67, 72
  calibration of, 72
Buchner funnel, 87
Bunge, Paul, 7

C

Cadmium sulphate, 163
Calcium, 135
  chloride, 21, 30, 34, 38
    tube, 16, 17, 32, 36, 37, 40–44, 46–50, 52, 55, 56, 57, 61, 62, 66, 67, 68
Calculations, 176–179
Capillary for weighing liquids, 62–63
Carbon, 1, 2, 5, 15–17, 28, 35, 49, 53, 56, 57, 64, 67, 69–72, 84
  dioxide, 38, 46, 47–53, 55, 70, 72, 74, 75, 76, 79–83, 84, 153, 161
  monoxide, 73, 74, 76
Carboxyl, 5, 148–150, 177
Carius, 1, 102, 115, 125–127
Caustic potash, 22, 72, 73, 74, 76, 87–89, 93
  soda, 19
Chlorine, 114
Cholesterin, 46
Chromium, 138
Cobalt, 135
Combustion tube, 23, 42, 46, 49, 58, 65, 66, 68, 71, 72, 83–85
    filling of, 25–31, 104, 105
  stand, 31–35
Copper, 2, 4, 28, 58, 66, 67, 83
  analysis, 69
  electrolytic deposition of, 138–144
  mortar, 32
  oxide, 25, 26, 28, 69, 76, 83, 85
  in preserved vegetables, 144–148
  spiral, 74
  sulphate, 102
Counterpoise, 12, 14
  aluminium, 14
  lead, 15
Crinis, Dr. Max de, 2
Cymene, 32, 65

D

Dachshund burner, 60
Distillation apparatus, 99
Distilled water, 109, 110
Donau, 7
Drying block, 60
Dubsky, Dr. Johann, 2, 15, 29, 30, 31, 79, 135
Dumas, 72, 75, 76, 78

E

Edlbacher, Dr. S., 2, 163
Eigner, Francis X., 32, 186
Emich, 1, 7, 28, 29, 108
Ether, 130
Ethoxyl, 150–154, 177
Ethyl chloride, 65
Euler, Prof. von, 5, 11

F

Filter tubes, 106–109, 128
Fischer, Prof., 5

G

Glycerine, 4, 22, 27, 45
Gold, 69, 135
  chloride, 163
Goldschmiedt, 2, 75
Gooch asbestos, 24, 25, 26, 87, 108
  crucible, 106

H

Halogen, 1, 2, 5, 16, 24, 28, 29, 54, 62, 64, 102–105, 111, 112–118, 125–127
  filtering tube, 128
Heating mortar, 31–35
Herzig, J., 69
Hydriodic acid, 155–156, 159–161, 163
Hydrogen, 1, 5, 15–17, 28, 34, 35, 44, 48, 49, 53, 57, 64, 67, 69–72, 84
  peroxide, 103, 119
  sulphide, 163

I

Indicators, 97, 124, 148
Iodine, 114

K

Kipp generator, 74, 75, 79–83, 84, 89, 110, 153, 161
Kjeldahl, 5, 73, 94–102, 145
Klemenc, A., 158
Kopfer, 24, 28
Kossel, Prof. A., 5
Krönig's cement, 21, 37
Kuhlmann, 7–15, 186

L

Lead acetate, 25
  chromate, 16, 25, 26, 28, 30, 51
  peroxide, 16, 24, 26, 28, 30, 31, 32, 48
    asbestos, 5, 26, 32, 51
  tubing, 18
Lieb, Hans, 2, 3, 4, 5, 104, 127–133, 157, 162
Lorenz, von, 128
Ludwig, 75

M

Magnesium, 135
Manganese dioxide, 17
Mariotte flask, 2, 16, 23, 29, 31, 38, 42, 43, 44, 47, 49, 50, 53, 54, 55, 58, 66, 67, 68, 72
Mercury, 87
  gas holder, 15, 16, 69, 71
Methoxyl, 150–154, 155, 156, 177
Methyl groups, 158–163, 177
Methyl iodide, 152
  orange, 148
  red, 97, 124
Micro azotometer, 72, 73, 74
  burner, 32, 162
  chemical balance, 7–15
  desiccator, 60
  Dumas method, 72–79
  Kjeldahl method, 94–102
  muffle, 5, 136
  Neubauer crucible, 14, 106, 119, 121

Micro—*continued.*
platinum crucible, 14
pipette, 98
Molecular weights, 164–172
Mortar, heating, 31
Muller, Ernst, 45

N

Neubauer crucible, 14, 106, 119, 121, 134
Nitric acid, 24, 25, 58, 110, 130
Nitrobenzene, 93
Nitro compounds, 28
Nitrogen, 1, 2, 16, 22, 28, 38, 54, 64, 72–79, 83–85, 158
as gas, 88–89
Nitroso compounds, 28
Nitrous oxide, 75

O

Oxyazo compounds, 28
Oxygen, 17–18, 20, 28, 66, 72, 74, 76, 177

P

Parnas, I. K., 99
Petridis, Dr. Georgios, 64
Petroleum, 32
Phenol, 34, 153
Phenol-phthalein, 148
Philippi, Dr. E., 3
Phosphomolybdate, 128, 129
Phosphoric acid, 128
pentoxide, 129
Phosphorus, 127–133
red, 155
Pilch, 101
Pinchcock, precision, 20
Platinum, 28, 58, 62, 68, 105–106, 118, 119
analysis of, 69
Poda, Heinrich, 2, 138
Porcelain beads, 104, 105
Potash, 17, 36
apparatus, 43
caustic, 73
Potassium, 135
chlorate, 17–18, 63, 64, 76
dichromate, 26, 68
phosphate, 128
Pressure regulator, 18–23, 30, 31, 48, 57, 65, 66, 168
regulations, 45–50
Pyridine, 162

R

Ramberg, Prof., 5
Regenerating block, 36, 61, 62, 108
Ripper, Dr., 4, 158
Rubber, 45, 49, 50

S

Sampling, 5, 6
Schöller, A., 30, 79, 82
Schrader, Prof., 45
Schwinger, Emil, 2, 102, 173
Silver, determination of, 69
halide, 103, 109, 115
iodide, 161
metallic, 16, 27, 28, 135
nitrate, 112, 152, 156, 157
wool, 23, 26
Soda-lime, 72
apparatus, 30, 49, 70
tube, 28, 37, 41, 43, 49, 50, 52, 53, 54, 55, 56, 65, 66, 67, 68
Sodium, 135
ammonium phosphate, 129
bicarbonate, 113
bisulphite, 103, 111, 112
carbonate, 110, 130
hydroxide, 124
sulphide, 102
Sprengel pump, 62
Strauss, F., 3
Strebinger, Robert, 108
Sulphur, 1, 2, 16, 24, 28, 54, 64, 103, 118–124, 125–127
dioxide, 111
Sulphuric acid, 32, 69, 103
chromic acid, 25
Sulphurous acid, 103

T

Tablet press, 169–171
Tablets, 169–171
Thiosulphate, 100, 102
Tube, absorption, 39–42, 53, 56, 58, 65, 71, 72
  combustion, 23, 42, 46, 49, 58, 65, 66, 68, 71, 72, 83–85
  filter, 106
  soda-lime, 28, 37, 41, 43, 49–56, 65, 68
Tubing, 17–18, 22
  indiarubber, 45, 49, 50, 53, 54, 57, 66, 82
  lead, 18

U

U tube, 21, 22, 26, 27, 52, 66, 72
  calibration of, 23
Universal filling, 16, 30

W

Wagner, R., 99
Weighing, 8–15, 58, 61, 62, 65, 120, 121
  bottle, 59
  tubes, 88
Widmar, Prof., 5
Wieland, Prof., 5
Willenberg, Hertha, 45
Willstätter, 5
Wine, 4
Wohack, Franz, 158
Wostall, Dr. Hans, 101

Z

Zero point deflection, 11, 13, 65
Zima, Dr., 5, 34
Zinc, 74

PRINTED IN GREAT BRITAIN BY THE WHITEFRIARS PRESS, LTD., LONDON AND TONBRIDGE.

UNIVERSITY OF ILLINOIS-URBANA
3 0112 067996394